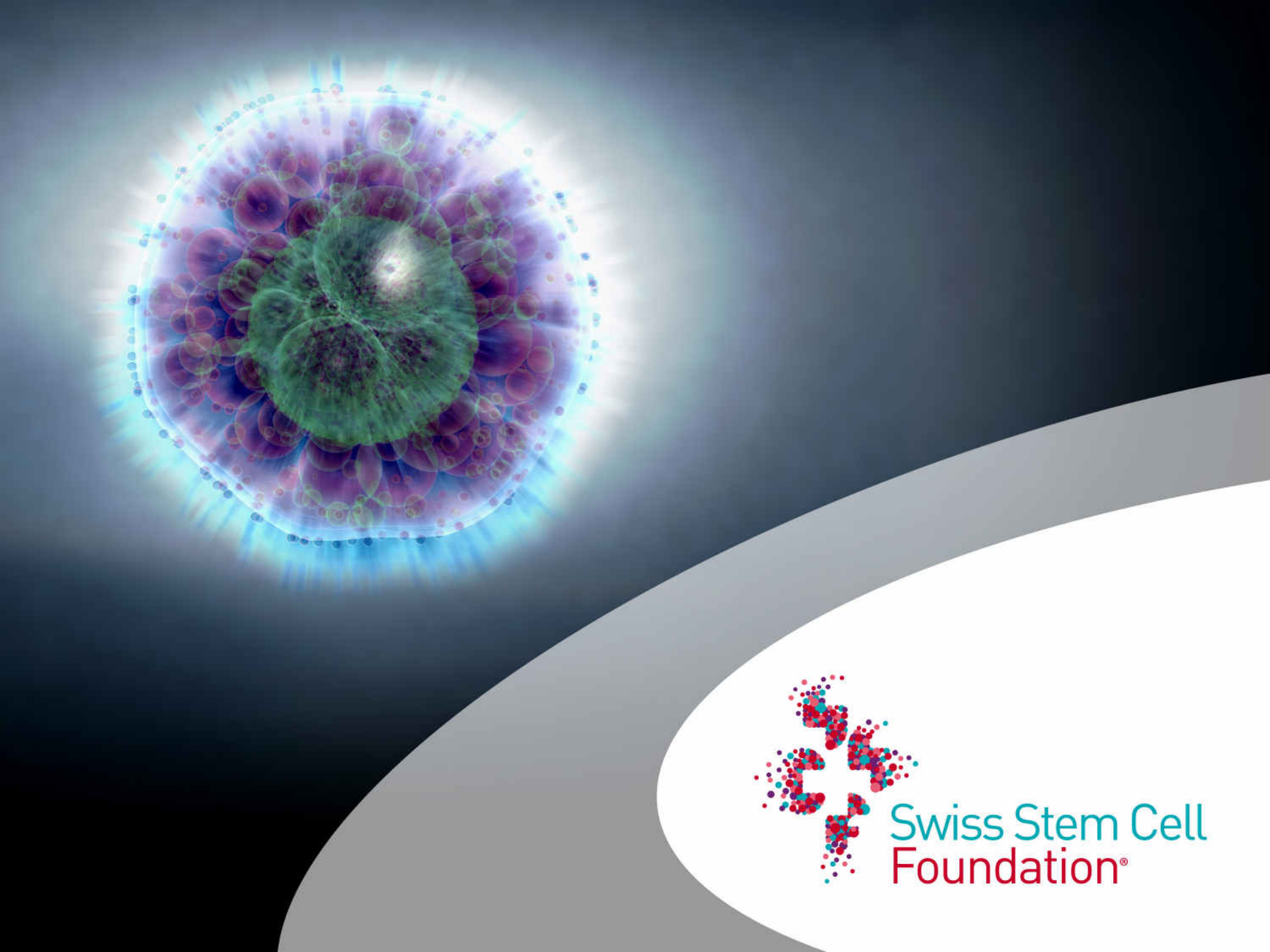
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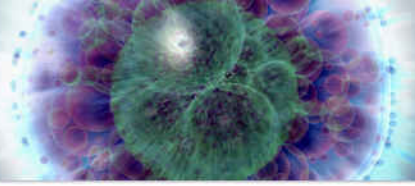
Expanding Adipose-Derived Mesenchymal Stem / Stromal Cells *In Vitro* for Stem Cell Therapies

Christian Caprara, PhD
Swiss Stem Cell Foundation

iCAST 2016

Zürich, 31.05.2016



- Adipose-derived mesenchymal stem / stromal cells (ASCs)
- Clinical-grade production of ASCs for cell therapy
- Cell expansion systems
- Product characterization and quality controls
- Quantum Cell Expansion System vs. flasks: yield and quality
- Conclusions and outlook

Adipose-Derived Mesenchymal Stem / Stromal Cells (ASCs)

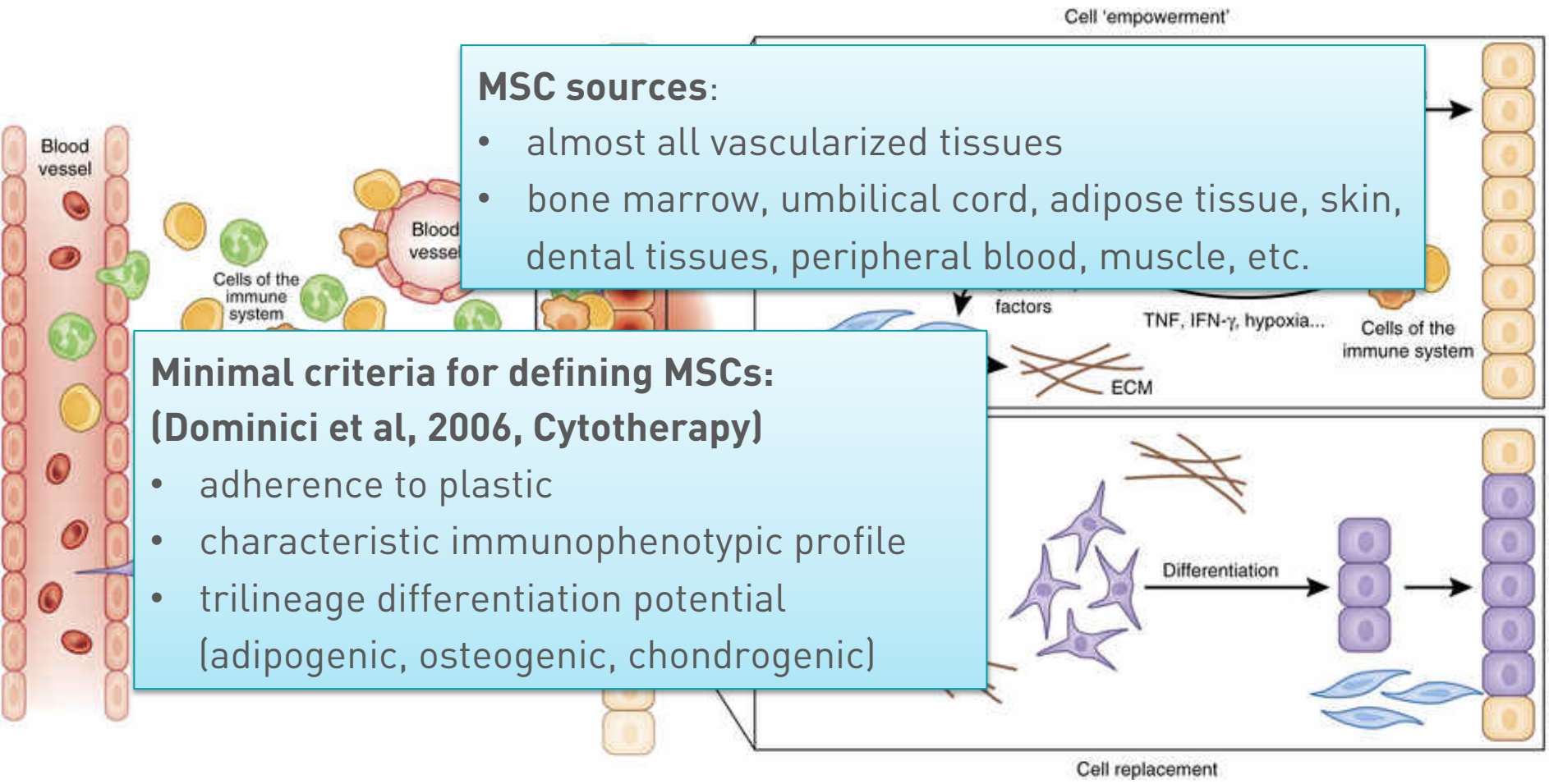
Mesenchymal Stem/Stromal Cells (MSCs)

MSC sources:

- almost all vascularized tissues
- bone marrow, umbilical cord, adipose tissue, skin, dental tissues, peripheral blood, muscle, etc.

Minimal criteria for defining MSCs:
(Dominici et al, 2006, Cytotherapy)

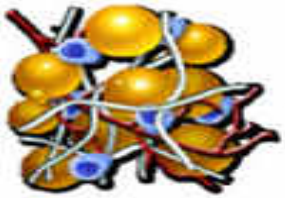
- adherence to plastic
- characteristic immunophenotypic profile
- trilineage differentiation potential (adipogenic, osteogenic, chondrogenic)



Wang et al, 2014, Nature Immunology

ASCs Are Found in the Stromal Vascular Fraction (SVF)

LIPOASPIRATE

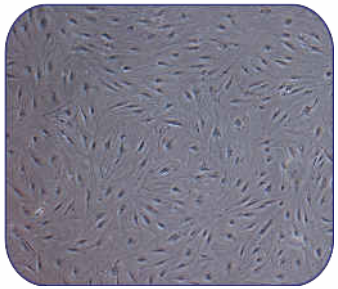


ENZYMATIC DIGESTION

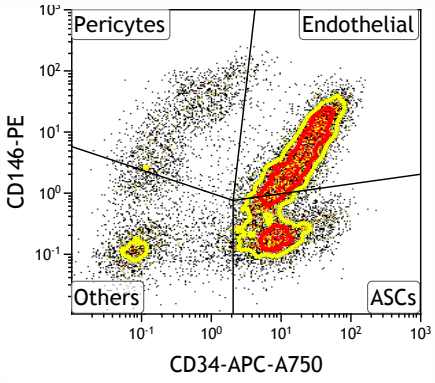
SVF

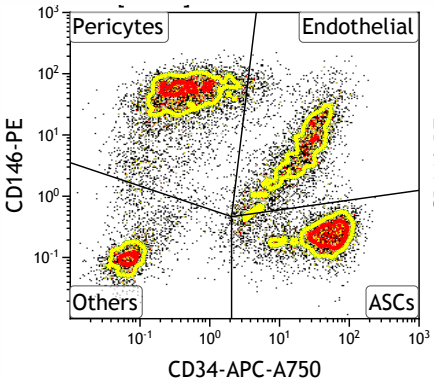
CELL CULTURE

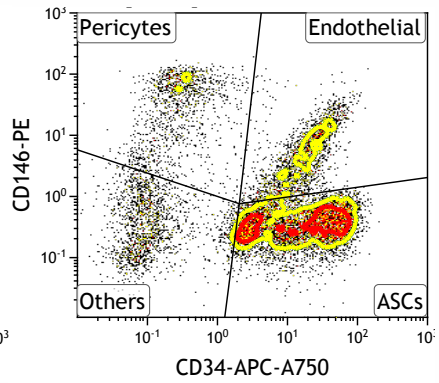
Adherent cells: ASCs



FLOW CYTOMETRY







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Cell Therapy with MSCs

Injury Healing

liver cirrhosis

liver failure

periodontal tissue defects

diabetic critical limb ischemia

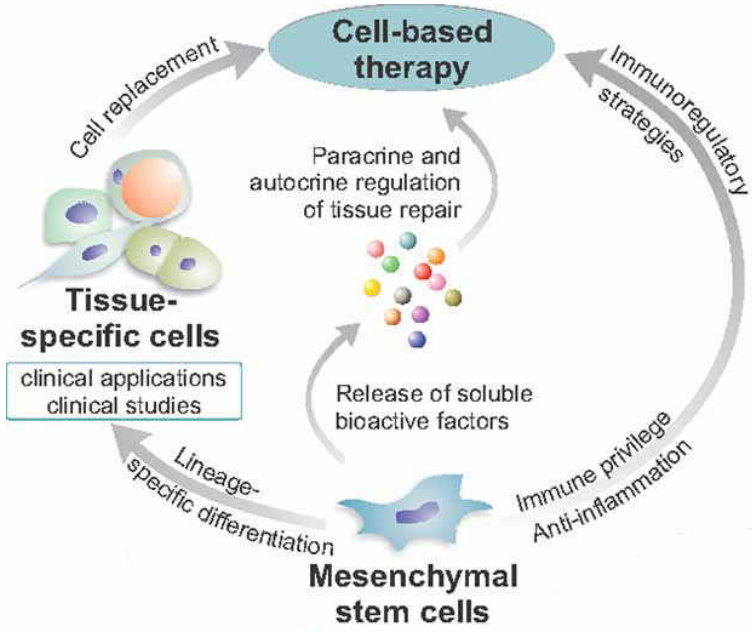
bone damage caused by osteonecrosis

burn-induced skin defects

myocardial infarction

cornea damage

brain and spinal chord injury



Tam and Pera, 2013. Bioessays

Immune Disorder Therapy

graft-versus-host disease

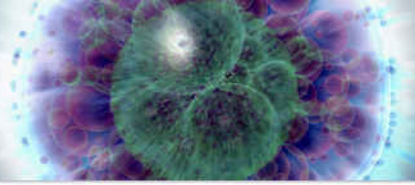
systemic lupus erythematosus

Crohn's disease

multiple system atrophy

multiple sclerosis

amyotrophic lateral sclerosis



Clinical-grade Production of ASCs for Cell Therapy

Cell Expansion Principles

- Clinical needs: 0.5 to 5 x 10^6 ASCs/Kg body weight (30 – 400 x 10^6 ASCs)
- 40 population doublings before entering senescence
  - maximum 20 population doublings for cell therapy (Prockop et al. 2010 Cytotherapy; Sensebe et al. 2011. Hum Gen Ther)

➢ **safety and efficacy** = functional ASCs that are safe and retain their therapeutic properties

➢ **develop a bioprocess in a well-defined environment**

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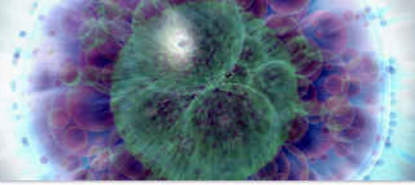

Product Characterization and Quality Controls

- Major challenge: to develop scalable manufacturing process while **maintaining critical quality parameters**
- Applying GMP to the manufacture of living biological drugs is not straightforward
  - GMP guidelines were designed for chemical manufacturing
  - Cell culture-based protocols are more complex than small molecule synthesis

➢ Product definition is more complicated

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Product Characterization and Quality Controls

Identity

- The product contains the intended cellular components
  - Immuno-phenotyping
  - CFU-F
  - Morphological appearance

Purity

- The product does not contain contaminant cell types or process reagents
  - Immuno-phenotyping

Safety

- The product is not contaminated with microbes and does not have tumorigenic potential
  - Sterility
  - Non-pyrogenic
  - Tumorigenicity/ Genomic stability (karyotiping)

Potency

- The product possesses biological functions relevant to treating the intended clinical indication
  - Differentiation
  - Immuno-supression
  - Release of bioactive molecules

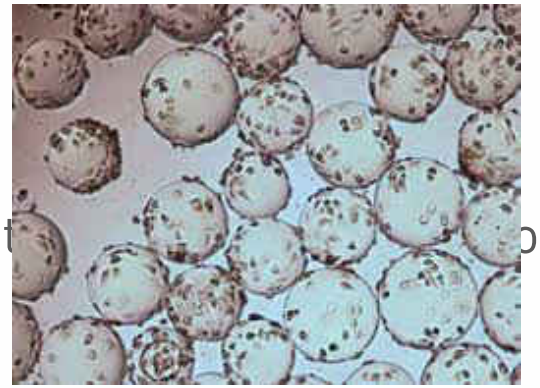
Cell Culture Systems

- **"Classic" T-flasks**
  - \+ cost-effective
  - \+ good gas exchange
  - – labor-intensive
  - – low yield per flask (max 300 cm^2)
  - – not fully closed (class A cabinet required)

- **Multilayer vessels** such as Cell STACKS (Corning) or Cell Factory (Thermo Fisher)
  - \+ less labor-intensive than T-flasks
  - \+ high yield possible (up to 25'280 cm^2)
  - – monitoring and harvest difficulties
  - – not fully closed (class A cabinet required)

Cell Culture Systems

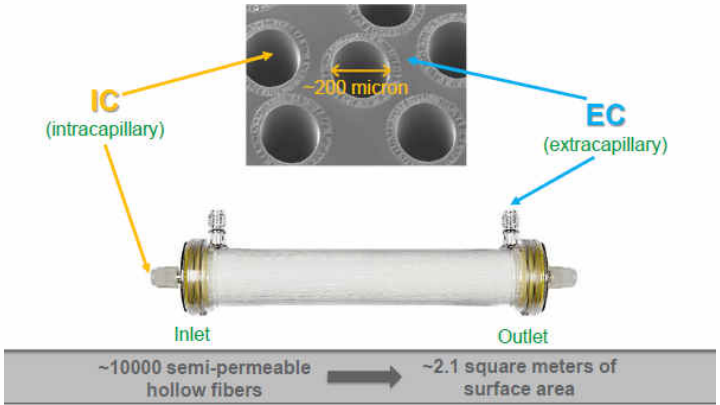
- **Closed automated devices**
  - + la e area t
  - + sa n
  - + si inocula vest ol of cu
  - + all class C
  - – can be more expensive for “small scale”



- **Bioreactors**
  - Multiplate (e.g. Pall Life Sciences), up to 122,400 cm^2
  - Hollow-fiber (e.g. Terumo BCT), up to 21’000 cm^2
  - Microcarrier (e.g. GE Lifesciences)

Quantum Cell Expansion System (Terumo BCT)

- **Hollow-fiber bioreactor**
  - 11,500 hollow fibers, 2.1 m^2 cell culture surface area
  - equivalent to the surface area of 120 T175 flasks
- Closed system
- Automated inoculation, harvesting, and control of temperature
- Perfusion system: cell feeding and waste removal
- Normoxic or hypoxic cell culture



Lactate Monitoring for Cell Number Prediction

- Within the bioreactor it is not possible to visualize cells
  - Cell number prediction though (daily) measurements of lactate concentration
    - lactate production is proportional to cell number

7 days
0.98 mmol/L

11 days:
1.22 mmol/L

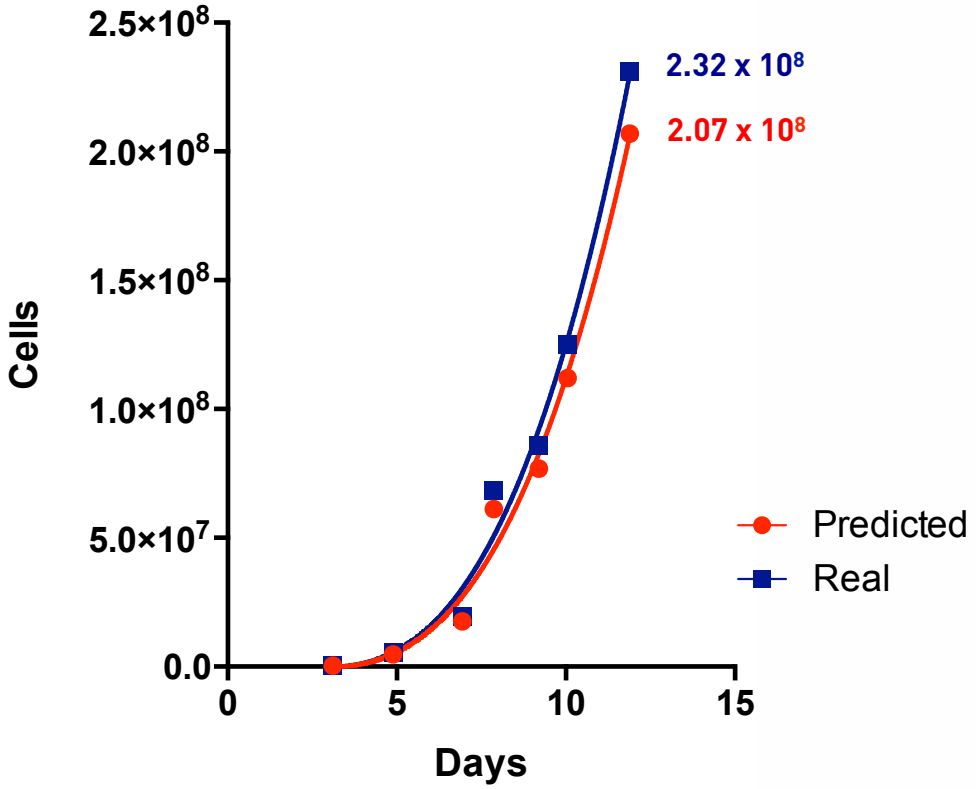
13 days:
4.42 mmol/L

  - Experimental determination (in flask culture) of the **maximal lactate production (mmol) per cell per day** (reference value for cell number prediction)

Quantum vs. Flasks

Yield

Lactate Measurements for Cell Number Prediction



- Seeded 9.6 x 10^6 ASCs
- 5% human platelet lysate (hPL) in IMDM:F12
- 21% O_2

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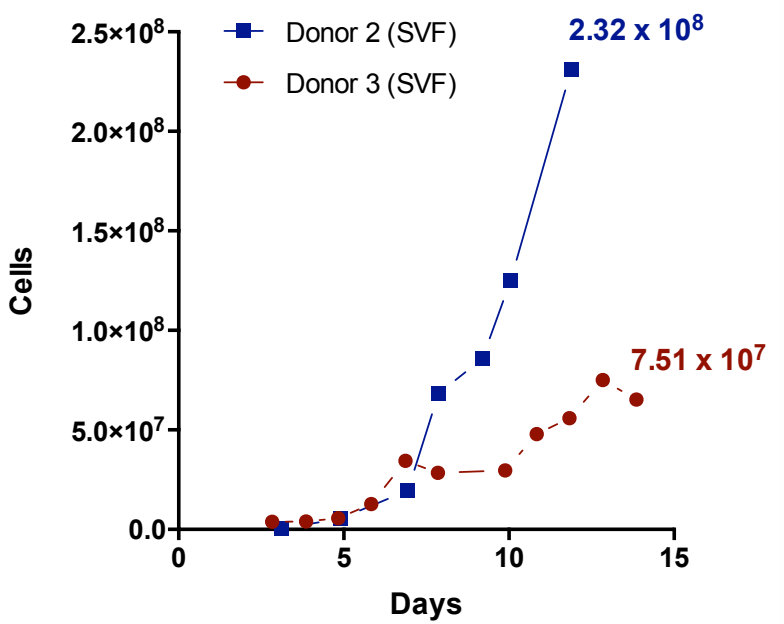
Donor-dependant Yield Variability

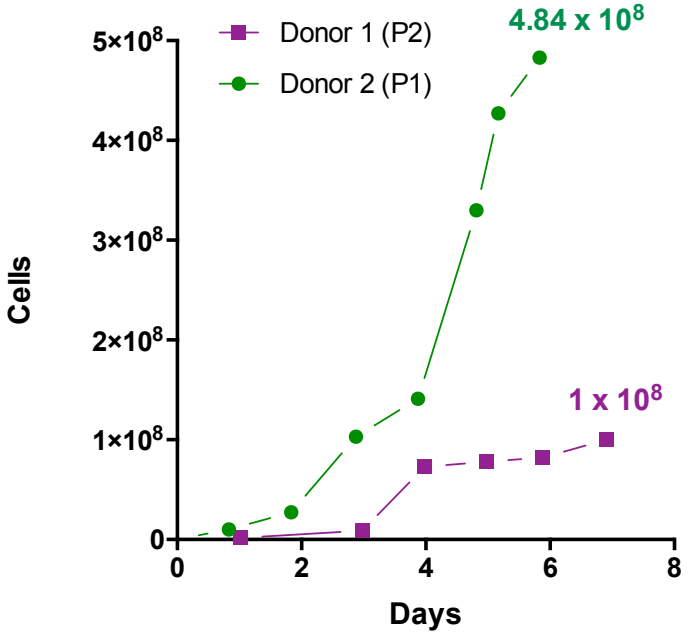
SVF (p=0)

5% hPL in IMDM:F12



pre-selected ASCs (p>1)

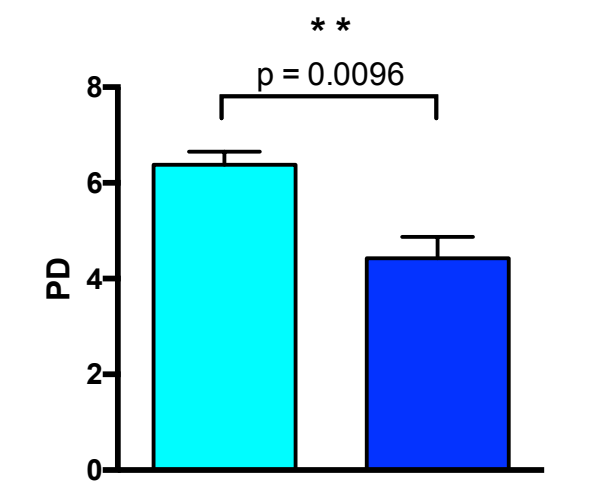
5% hPL in IMDM:F12



	Seed	Harvest	Culture Duration (days)	PD (cumulative)
Donor 2 (SVF)	9.60 x 10^6	2.32 x 10^8	11.90	5.18
Donor 3 (SVF)	1.05 x 10^7	7.51 x 10^7	13.80	3.43
Donor 2 (ASC, P1)	2.00 x 10^7	4.84 x 10^8	5.83	10.36
Donor 1 (ASC, P2)	1.00 x 10^7	1.00 x 10^8	6.90	6.76

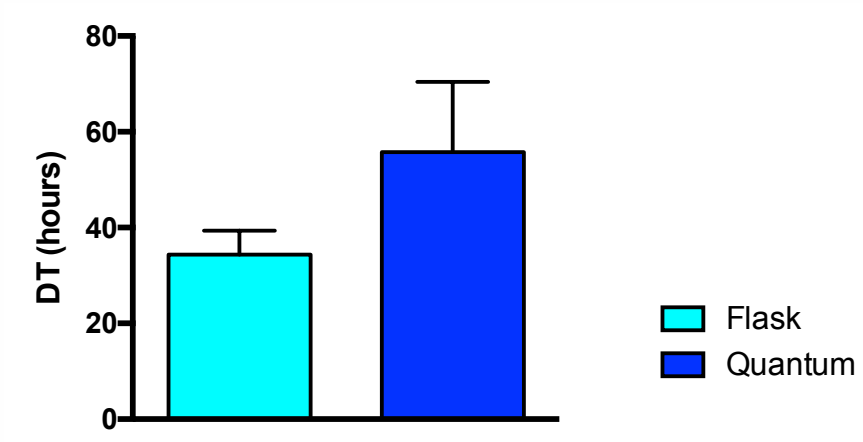
Higher ASC Proliferation in Flasks

Population Doublings



	Flask			Quantum		
	Mean	SEM	N	Mean	SEM	N
PD	6.378	0.269	4	4.420	0.449	4

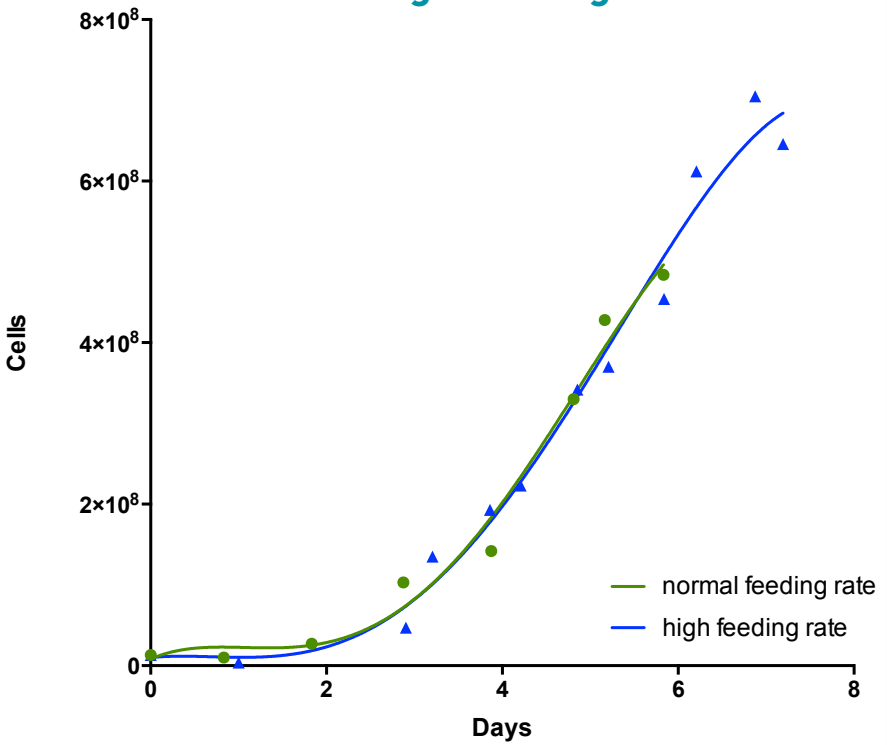
Doubling Time



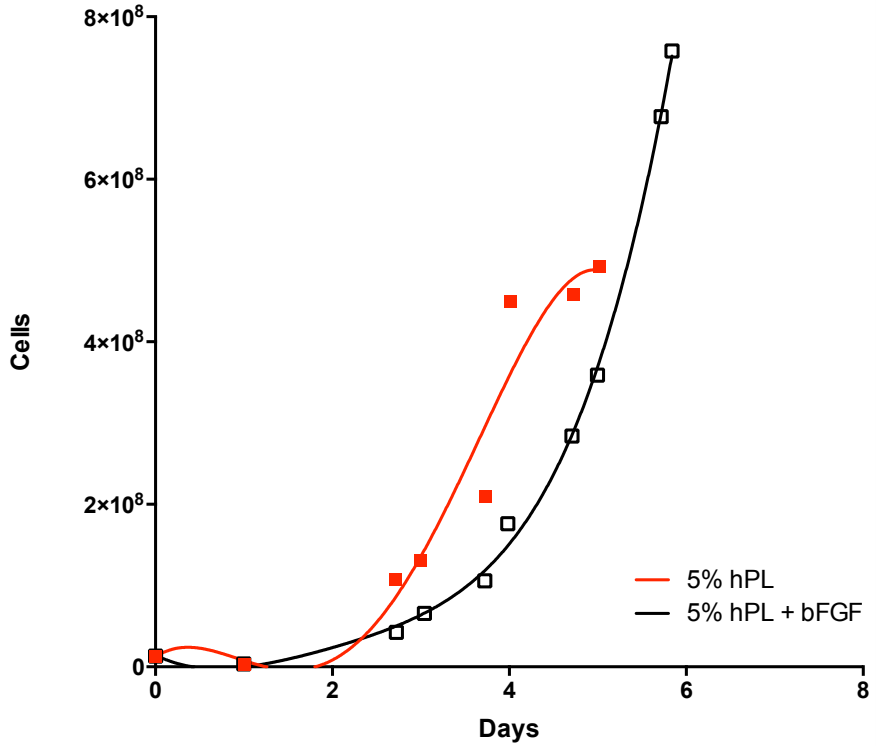
	Flask			Quantum		
	Mean	SEM	N	Mean	SEM	N
DT	34.360	5.006	4	55.735	14.706	4

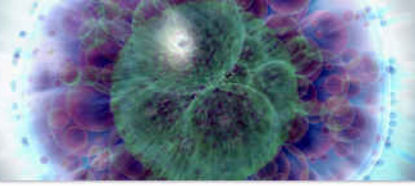
Medium and Feeding Rate Optimization

High feeding rate



+ bFGF





Quantum vs. Flasks

Quality

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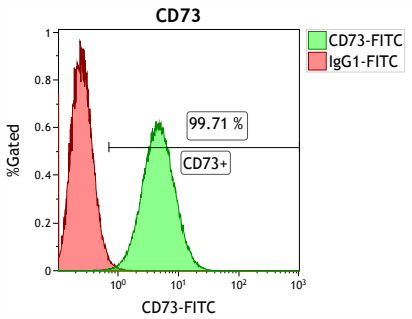
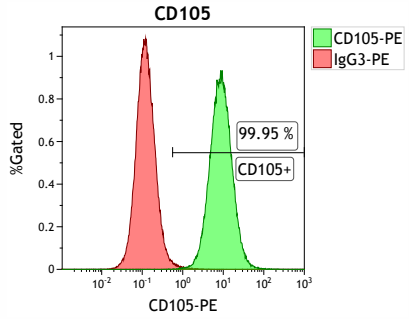
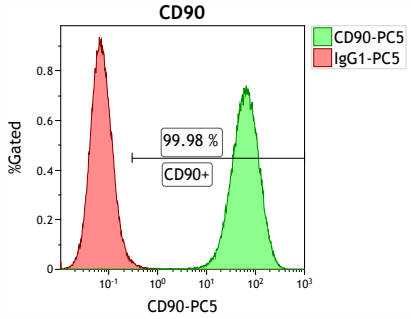
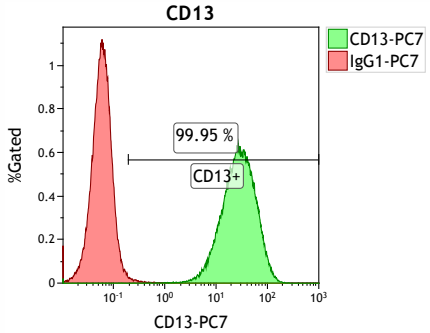
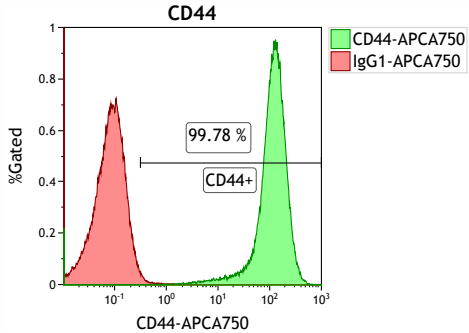
Immunophenotyping

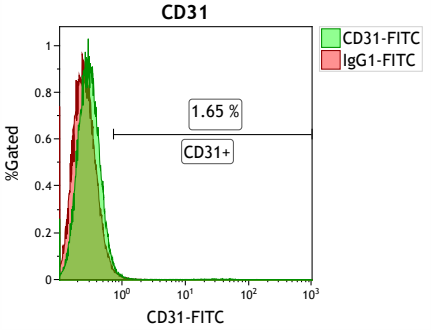


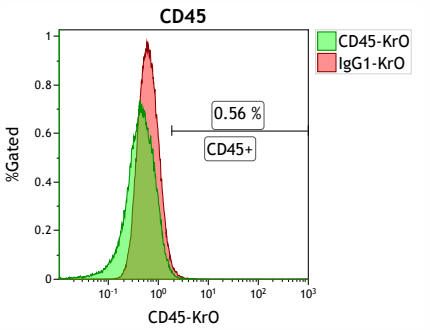


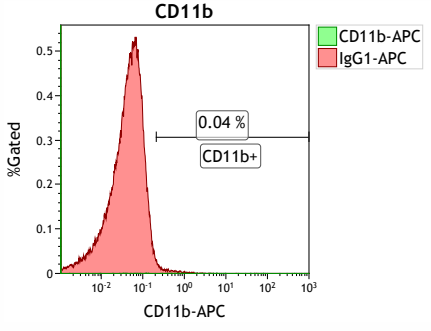






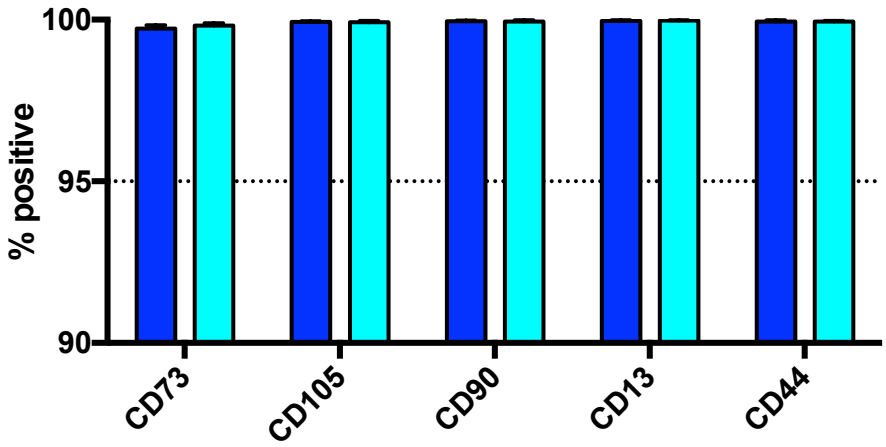




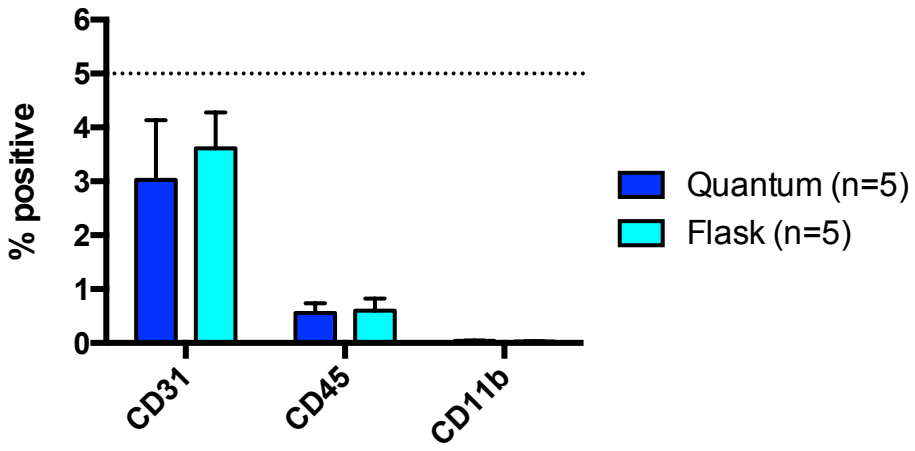


Immunophenotyping

MSC Positive Markers

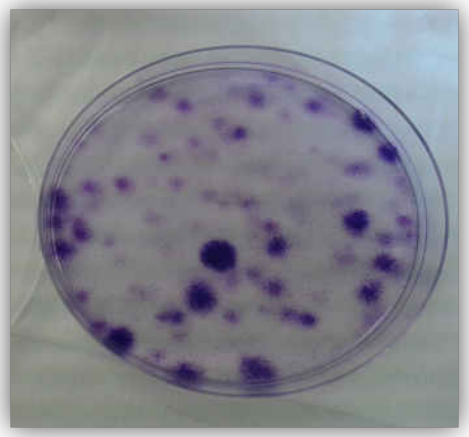


MSC Negative Markers



Colony-Forming Unit Fibroblast (CFU-F) Assay

- Acceptance criteria for CFU-F
  (Bourin *et al.* 2013, Cytotherapy)
  - "fresh" SVF: > 1%
  - pre-selected ASCs: > 5%



	SVF (CC)	SVF (0207)	pre-selected ASCs (CC, p1)	pre-selected ASCs (ML, p2)
Before seeding	n/a	0.65%	7.80%	1.8%
At harvest (Quantum)	7.80%	14.5%	8.90%	4.05%
At harvest (Flask)	8.20%	17.3%	7.65%	4.15%

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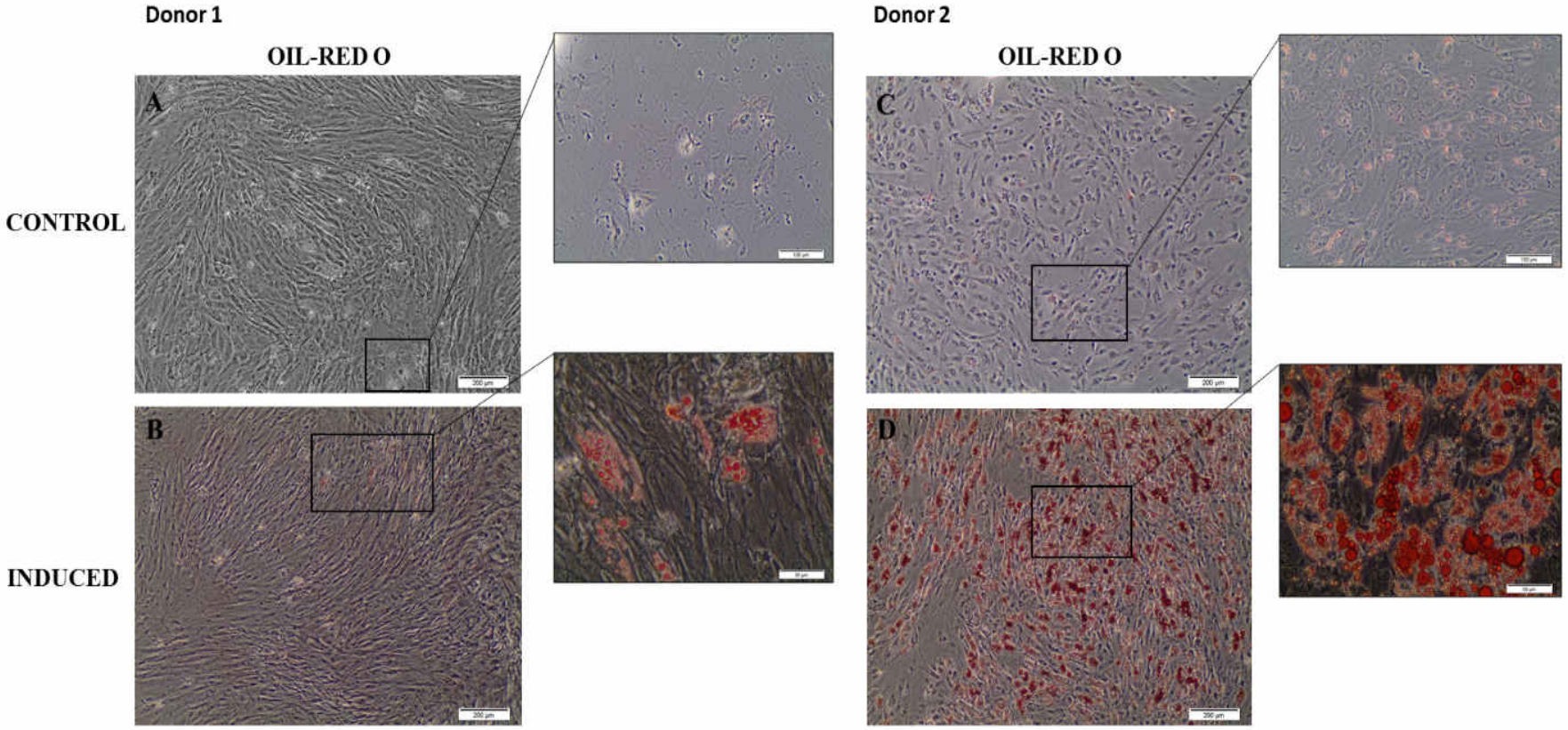
Adipogenic Differentiation Potential

Quantum



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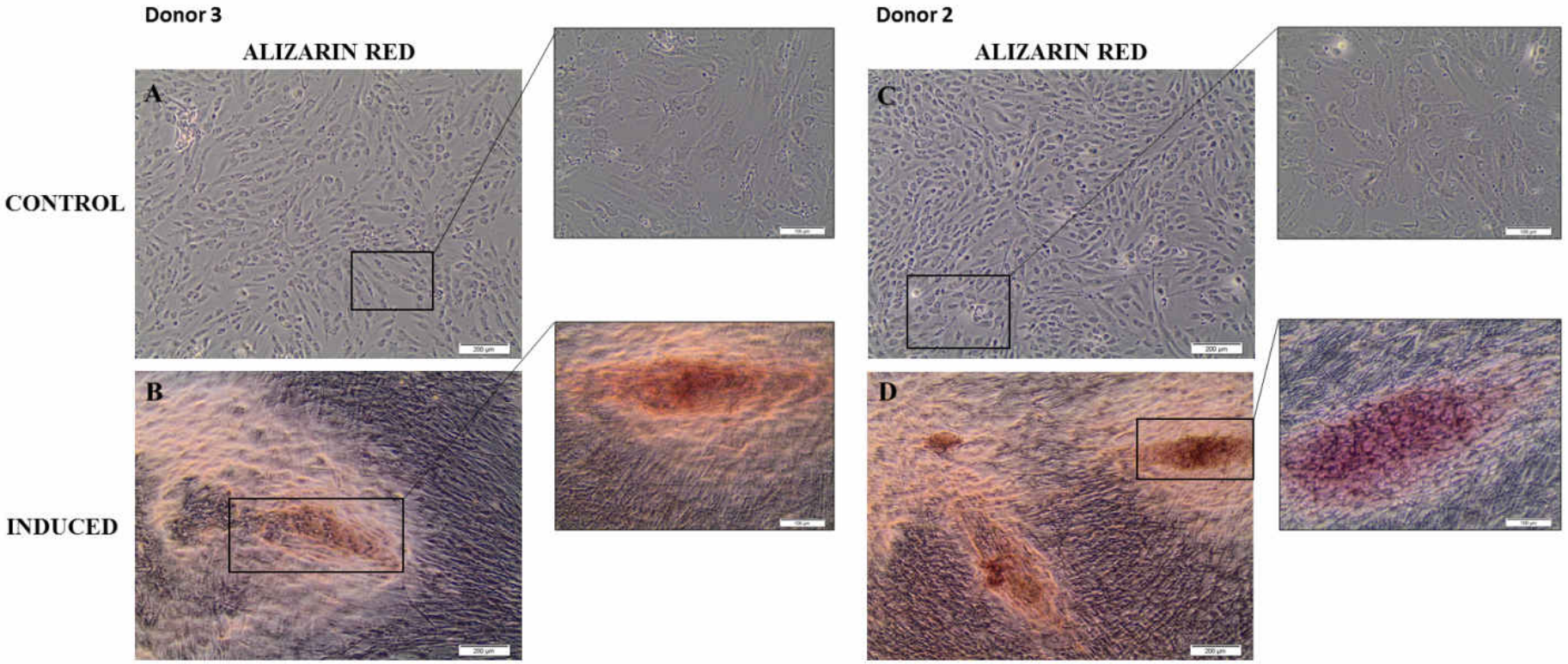
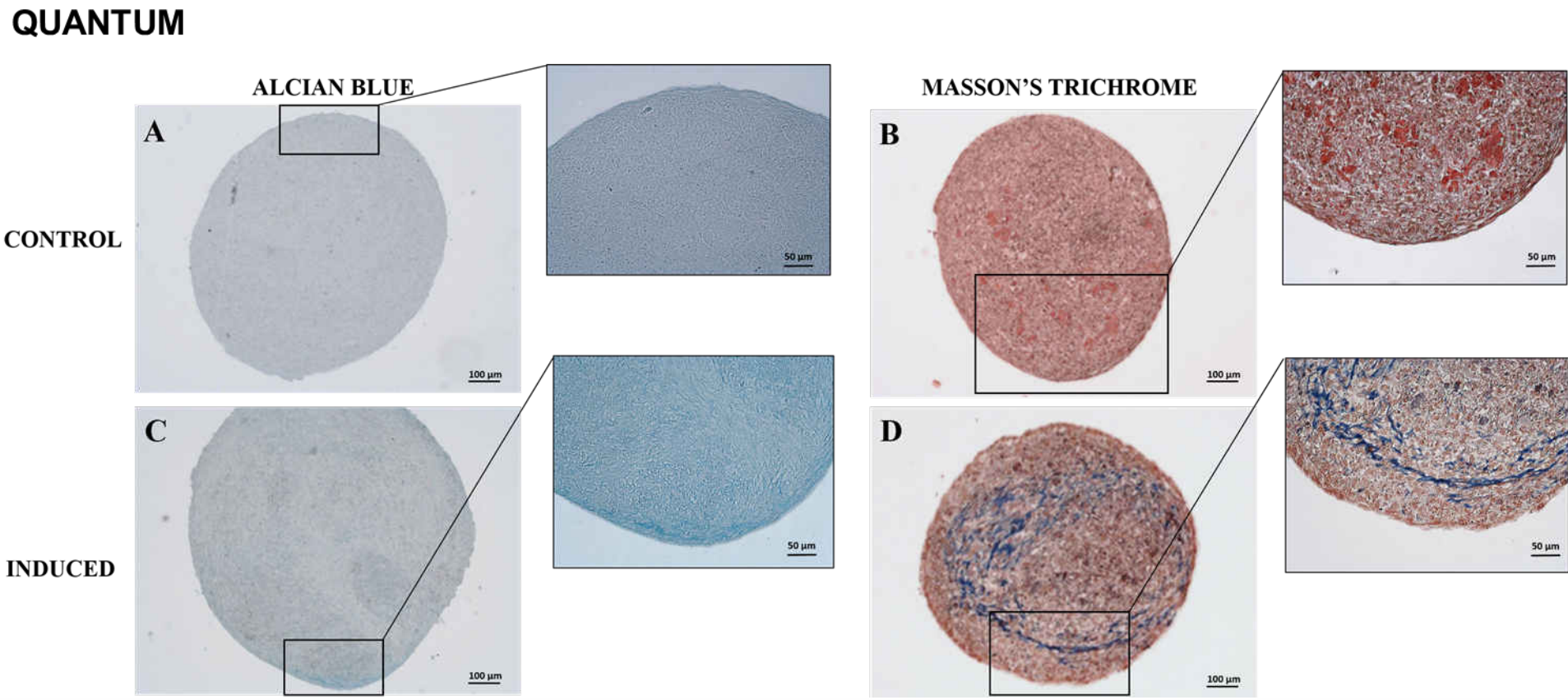
Osteogenic Differentiation Potential

Quantum



Chondrogenic Differentiation Potential



Courtesy of Prof. Gornati and Prof. Bernardini

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DNA Damage/Repair, Apoptosis, Cell Cycle (RT^2 Profiler PCR Arrays, Qiagen)

ATM / ATR Signaling

ATM, ATR, ATRIP, BARD1, BRCA1, CDC25A, CHEK1, CHEK2 (RAD53), CSNK2A2, FANCD2, H2AFX, HUS1, MDC1, PARP1 (ADPRT1), RAD1, RAD17, RAD50, RAD9A, RBBP8, RNF168, RNF8, SMC1A (SMC1L1), TOPBP1, TP53 (p53).

DNA Damage & Repair

Nucleotide Excision Repair (NER): CDK7, DDB1, DDB2, ERCC1, ERCC2 (XPD), LIG1, NTHL1, OGG1, PCNA, PNKP, RPA1, SIRT1, TP53 (p53), XPA, XPC.

Base Excision Repair (BER): APEX1, FEN1, LIG1, MBD4, MPG, NTHL1, OGG1, PARP1 (ADPRT1), PCNA, TP53 (p53), UNG, XRCC1.

Mismatch Repair (MMR): ABL1, EXO1, MLH1, MLH3, MSH2, MSH3, PCNA, PMS1, PMS2, TP73.

Double-Strand Break (DSB) Repair: ATM, BLM, BRCA1, CHEK1, H2AFX, HUS1, LIG1, MDC1, MLH1, MRE11A, NBN (NBS1), PRKDC, RAD50, RAD51, RPA1, TP53BP1, XRCC2, XRCC6 (G22P1).

Other DNA Repair Genes: ATR, ATRIP, ATRX, BARD1, BRIP1, CHEK2 (RAD53), CIB1, CRY1, FANCA, FANCD2, FANCG, GADD45A, GADD45G, RAD1, RAD17, RAD18, RAD21, RAD51B (RAD51L1), RAD9A, RBBP8, REV1 (REV1L), RNF168, RNF8, SMC1A (SMC1L1), SUMO1, TOPBP1, XRCC3.

Apoptosis

ABL1, ATM, BARD1, BAX, BBC3, BRCA1, CDKN1A (p21CIP1, WAF1), CHEK2 (RAD53), CIB1, CSNK2A2, PPP1R15A (GADD34), PRKDC, RAD21, RAD9A, SIRT1, TP53 (p53), TP73.

Cell Cycle

ATM, ATR, ATRIP, CDC25A, CDC25C, CDK7, CDKN1A (p21CIP1, WAF1), CHEK1, CHEK2 (RAD53), DDIT3 (GADD153, CHOP), MAPK12 (P38GAMMA), MCPH1, MDC1, PPM1D, PPP1R15A (GADD34), TP53 (p53), TP73.

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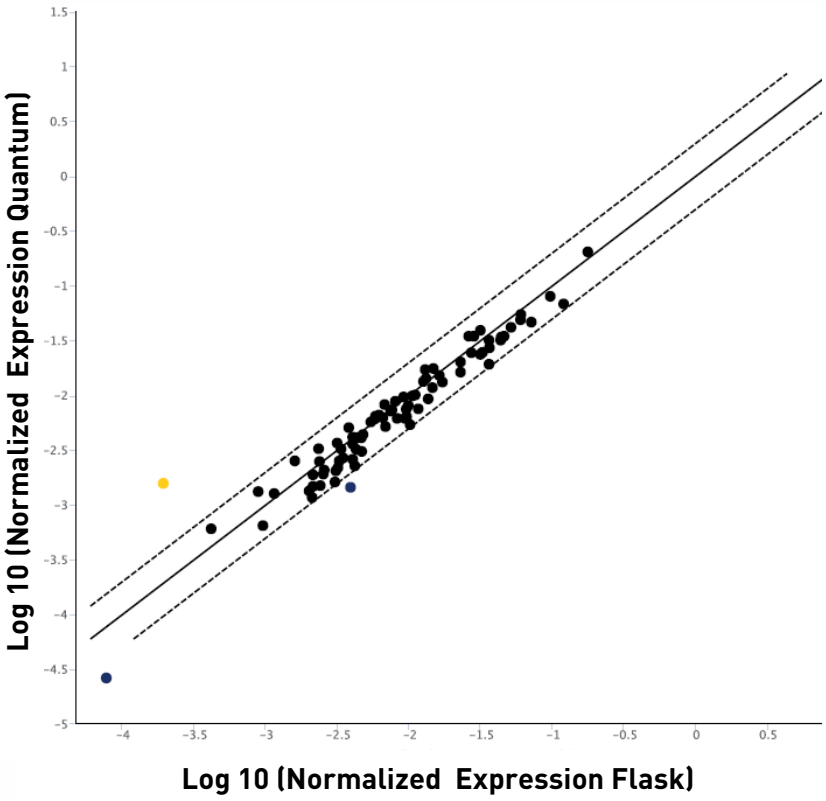
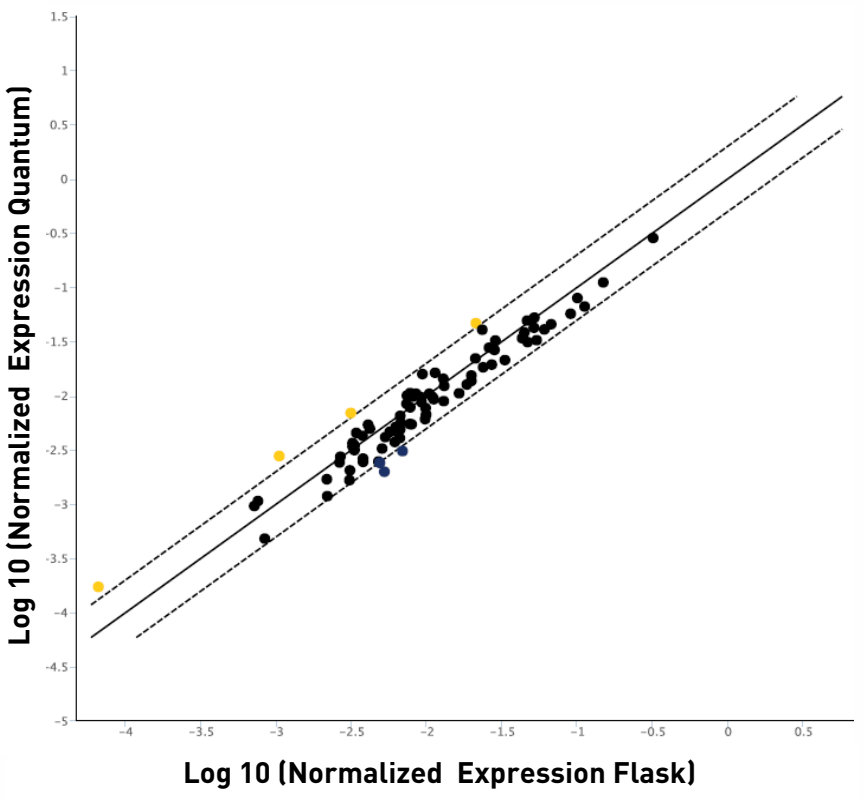
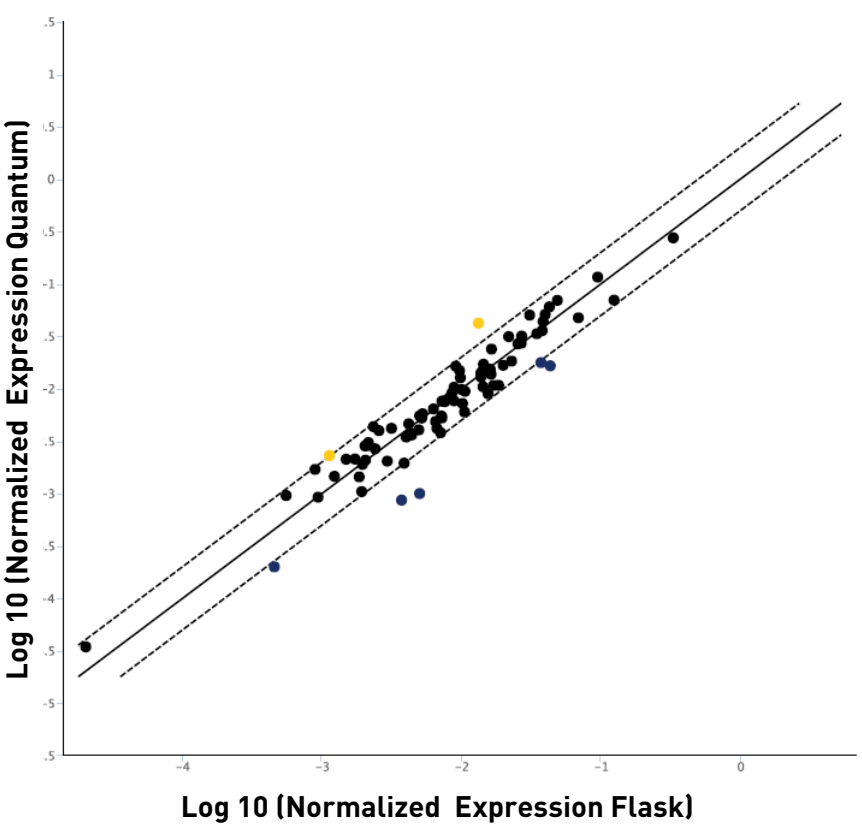
DNA Damage/Repair, Apoptosis, Cell Cycle

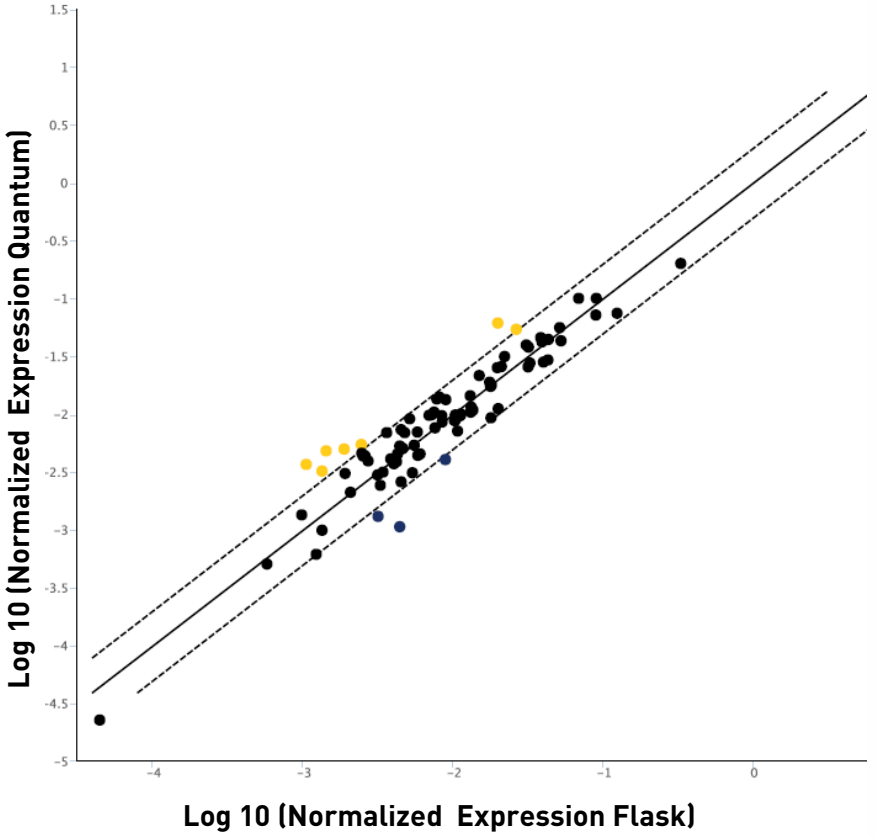




- upregulated
- downregulated
- unchanged

DNA Damage/Repair, Apoptosis, Cell Cycle





- upregulated
- downregulated
- unchanged

Conclusions and Outlook

- ASCs can be expanded in the Quantum
  - Yield variability: donor and culture conditions
  - Bioprocess transition (flask to Quantum) needs optimization
- The quality of ASCs expanded in the Quantum is comparable to flask-expanded cells
  - Identity and purity: confirmed
  - Safety: need for further genomic stability testing, tumorigenicity assay, sterility and endotoxin testing
  - Potency: “classical” differentiation confirmed, need for therapy-oriented potency assays

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Acknowledgments

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Brent Rice, PhD

Università degli Studi dell'Insubria

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Prof. Giovanni Bernardini, PhD

Thank You for Your Attention!

Questions?