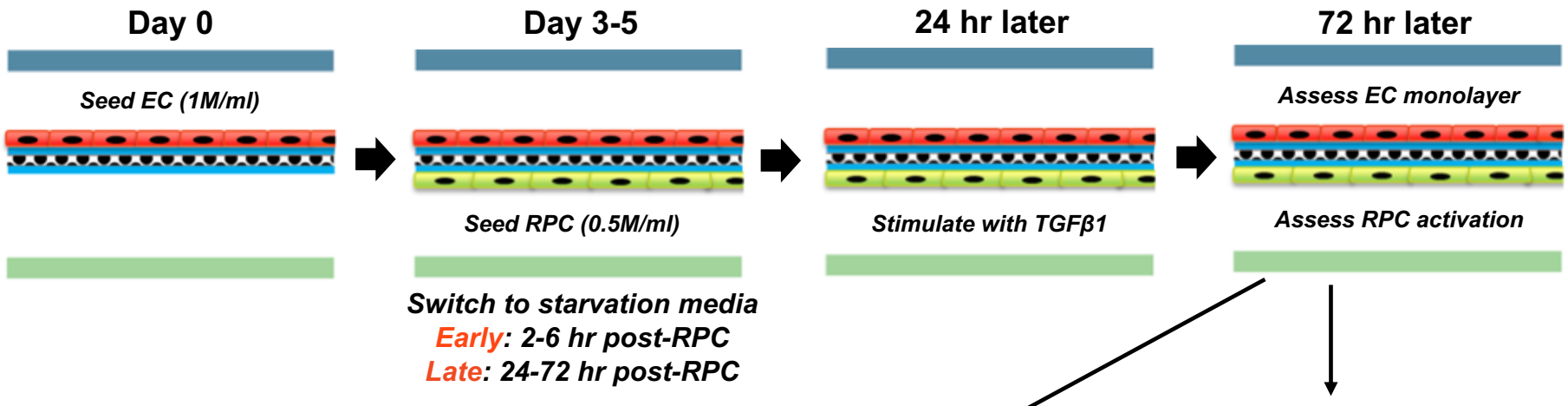
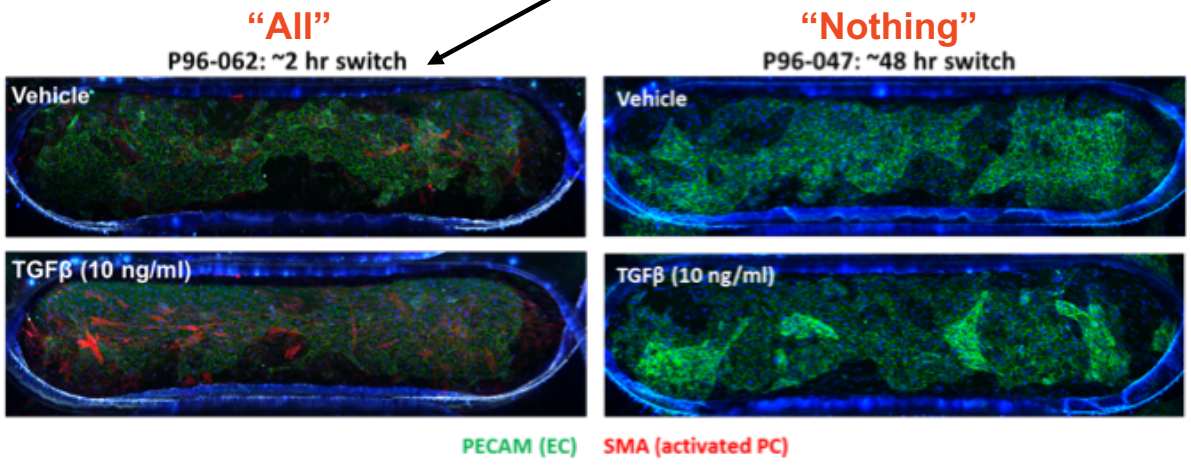
RNA-seq Pilot: Overview

Observation: "All or nothing" response based on timing of starvation after pericyte seeding



Questions addressed:

- **Feasibility/ Compatibility**
- **Biological variability**
- Signaling differences between early and late starvation?
- Differences in mono- vs. co-culture RPC activation?



RNA-seq Pilot: Bio Replicate Summary

Replication/ Minimizing variability: Same cell and reagent stocks used; Procedures carried out on same day/time as other replication experiments; Same personnel (CW for all cell work, MTR for all RNA). Green bio replicates to be used for pilot study

Condition	Expected	1	2	3	4
RPC mono (control)	No activation	✓	✓	x Few/ no cells	✓
RPC mono (+ TGFβ1)	Activation (SMA+)	✓+	✓	x Few/ no cells	✓
EC mono (starved)	Patchy monolayer	✓	✓	✓-	✓
Early starve co: EC	Patchy monolayer	✓	✓	✓	✓
Early starve co: RPC	Activation (SMA+)	✓+	✓+	✓	✓-
Late starve co: EC	Better monolayer	✓	✓-	✓	✓
Late starve co: RPC	No/less activation	✓+	✓-	✓+	✓

RNA-seq Pilot: Status Update

Optimize MVEC harvest for RNA extraction

Sufficient and high quality RNA can be obtained

Optimize/monitor MVEC coverage (don't proceed if poor) → Time course for expression of select genes to guide pilot → Run biological replicates (3 independent experiments) →

Robust MVEC lot identified

RNA collection at 5 hr and 24 hr post starvation

IF complete, used for pre-screen Luminex TBD

We are here

Pilot study: Variability, differences in mono- vs. co-culture activation →

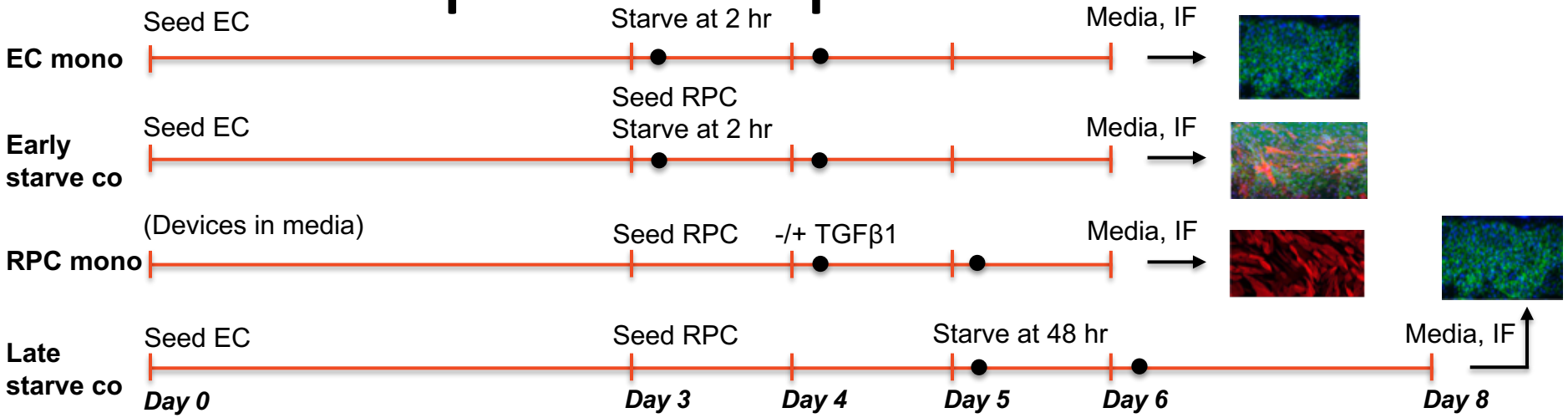
1+ technical replicates passed QC (41/42 samples)

Larger study: additional time points, adding TGFβ1

Parallel readouts will include IF, Luminex, which can be linked in PCA analysis

Go: Targets to pursue, validation by follow up experiments
No go: No obvious targets or too many, high variability

RNA-seq Pilot: Experimental Plan



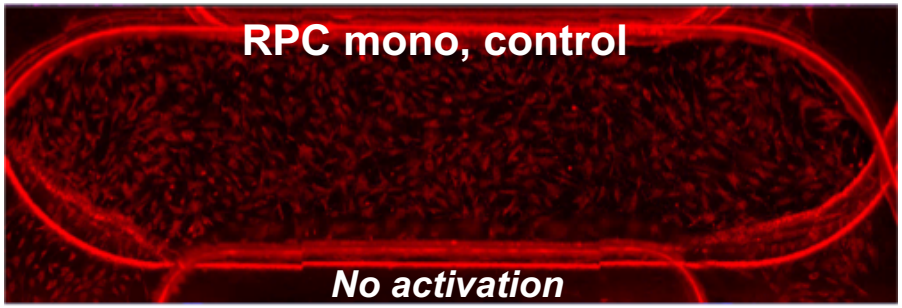
Harvest Time Post-Starve/Stim	5 hr	24 hr	
EC mono starve	EC	EC	
RPC mono (-/+ TGFβ1)	RPC (-)	RPC (-)	
	RPC (+)	RPC (+)	
Early starve co-culture	EC-co	EC-co	
	PC-co	PC-co	
Late starve co-culture	EC-co	EC-co	
	PC-co	PC-co	
N = 3 per condition	21	21	**42**

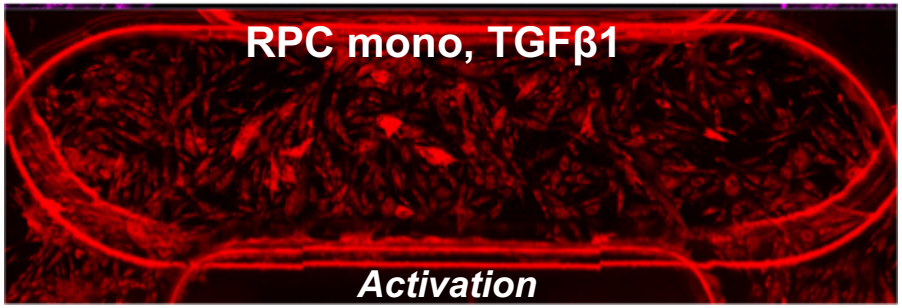
● *Indicates media, RNA collection*

Questions addressed:

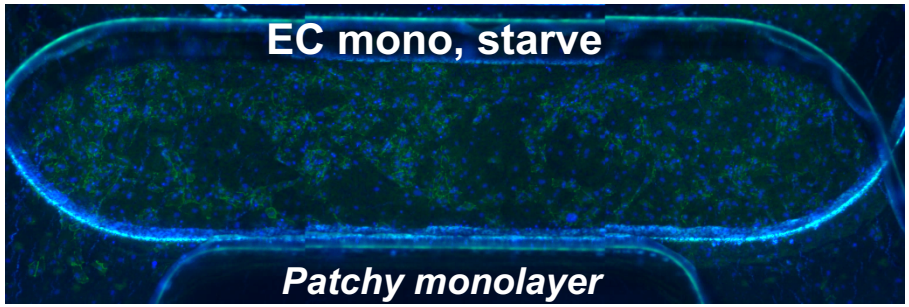
- **Feasibility/Compatibility with P96**
- **Biological variability**
- Signaling differences between early and late starvation co-cultures
- Inhibitory pathways/factors in late starvation model?
- Differences in mono- vs. co-culture RPC activation?

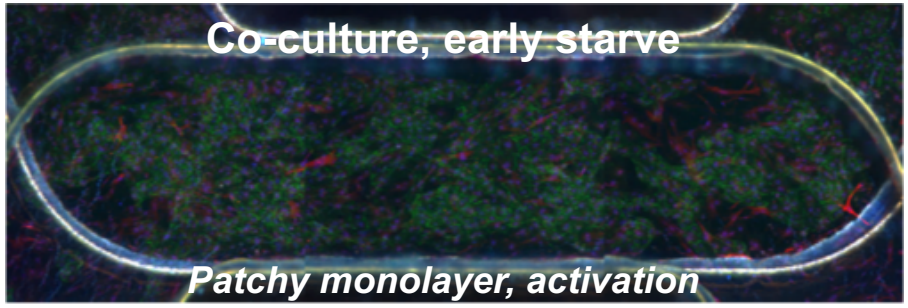
RNA-seq Bio Replicates: Representative IF

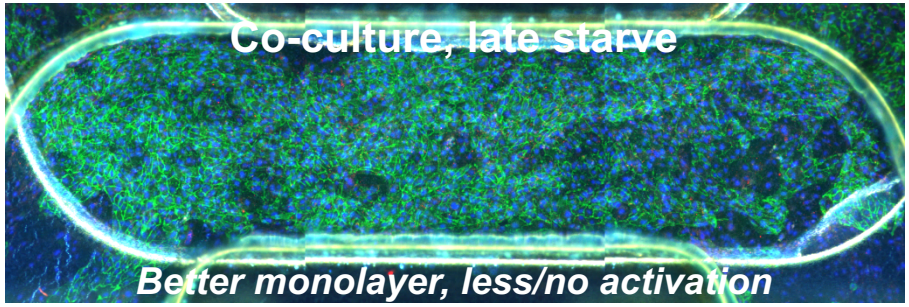




Hoechst
PECAM-1 (EC)
SMA (activated RPC)







RNA-seq Bio Replicates: QC Results

Sample	Time point	RINe	Conc. [pg/µl]	Total amount (ng)
EC mono bio rep 1	5 hr	9.1	1420	14.2
EC mono bio rep 1	24 hr	8.9	942	9.42
Early coculture EC bio rep 1	5 hr	8.9	1240	12.4
Early coculture EC bio rep 1	24 hr	9.1	2520	25.2
Early coculture RPC bio rep 1	5 hr	8.8	1700	17
Early coculture RPC bio rep 1	24 hr	9	2090	20.9
RPC mono - TGF bio rep 1	5 hr	9.5	1320	13.2
RPC mono + TGF bio rep 1	5 hr	8.4	1820	18.2
RPC mono - TGF bio rep 1	24 hr	9.2	571	5.71
RPC mono + TGF bio rep 1	24 hr	9	2640	26.4
EC late coculture bio rep 1	5 hr	7.3	3030	30.3
RPC late coculture bio rep 1	5 hr	8	2070	20.7
EC late coculture bio rep 1	24 hr	8.6	4630	46.3
RPC late coculture bio rep 1	24 hr	8.7	4430	44.3
EC mono bio rep 2	5 hr	8.4	8750	87.5
EC mono bio rep 2	24 hr	6.6	25300	253
EC early coculture bio rep 2	5 hr	7.9	11100	111
RPC early coculture bio rep 2	5 hr	8.8	3460	34.6
EC early coculture bio rep 2	24 hr	7.8	15400	154
RPC early coculture bio rep 2	24 hr	8.9	7400	74
EC late coculture bio rep 2	5 hr	7.7	6370	63.7
RPC late coculture bio rep 2	5 hr	9.3	1280	12.8
EC late coculture bio rep 2	24 hr	7.7	15900	159
RPC late coculture bio rep 2	24 hr	8.8	4480	44.8
RPC mono -TGF bio rep 2	5 hr	8.7	4420	44.2
RPC mono +TGF bio rep 2	5 hr	8.8	4520	45.2
RPC mono -TGF bio rep 2	24 hr	8.7	5560	55.6
RPC mono +TGF bio rep 2	24 hr	8	3630	36.3
EC mono bio rep 3	5 hr	9	2380	23.8
EC mono bio rep 3	24 hr	8.6	7580	75.8
RPC mono -TGF bio rep 3	5 hr	8.2	1200	12
RPC mono +TGF bio rep 3	5 hr	8.5	2180	21.8
RPC mono -TGF bio rep 3	24 hr	7.9	7640	76.4
RPC mono +TGF bio rep 3	24 hr	8	7460	74.6
EC early coculture bio rep 3	5 hr	7.6	12900	129
RPC early coculture bio rep 3	5 hr	8.2	3200	32
EC early coculture bio rep 3	24 hr	8.2	4160	41.6
RPC early coculture bio rep 3	24 hr	8.4	6730	67.3
EC late coculture bio rep 3	5 hr	7.1	6280	62.8
RPC late coculture bio rep 3	5 hr	7.6	30000	300
EC late coculture bio rep 3	24 hr	7.2	20400	204
RPC late coculture bio rep 3	24 hr	8	36500	365