

A HMGA1 TGIF1	88 33 48 48	40ng ADNB	Diss 12 48h C SREBF1 SREBF2 EIF4E TBX2 PPARGC1B	LL13 CSF2 TNF CD40LG LL1A	Diss 12 48h RHOA KRAS TGM2 IRS1 NCF1	D NUPR1	NF-α TGFB1 TGFB1 EGF FGF2 VEGFA HGF	x TGF-β/TNF-α 20 40 ng/mL PTGS2 FN1 TGM2 TGM2 TET2 TDAGE	E e b miR155HG miRLET7BHG 54miR600HG ***	F 300 - miR22HG - miR100HG
JUNB ELK3 FOSL2 PPARG NFKB2 JUN		MYC ELK3 MAFF NFKB2 RUNX1 SOX11	CREB1 MTPN SP1 STAT3 STAT1 RELA NFKB1 ECSIT DACH1 OTX2	TNFSF11 PRL CCL5 TNFSF12 IL3 SPP1 TSLP TNFSF13B IL17F	HRAF2 HRAS PIN1 PTGS2 STUB1 BRMS1 CAT GPX1 KAT2A PCYT1A	HMGB1 SMARCA4 ETS1 JUN PPRC1 GLI1 SP1 MTPN CREB1	TGFB3 AGT NRG1 AREG TGFB2 TGFB2 TGFA MSTN FGF7 HBEGF KITLG	KRAS SNCA PLA2G10 CYP1A2 TRAF2 NOS2 PLCE1 NOX4 TERT RAC1	Picka dold	- 0 100- With Disconsistion time (hr)
MAFF EGR1 ESRRA FOSL1 SOX3 RELB FOS		LMO2 ARNTL RELB ETV5 EGR2 FOSB HMGA2	REBB2 INSR IPMK RAF1 SRC EGFR MP2K4	II3 IL1B IFNG EDN1 FASLG IL5 IL5 IL6 THPO NAMPT CXCL12	RAF6 PLD2 RAC1 RNASEL RNF20 TERT TET2 TNFAIP3 ARG1 ITCH ACACB	JUNB FOXM1 EGR1 ZFP36 BCL6 KLF2 TAF4 MEOX2 OTX2	JAG1 BTC PGF EREG PDGFB ANGPT2 GRP GDNF NOG DKK1	GNAI3 ENPP2 SPTLC2 IL411 KL ADA TNFAIP3 DICER1 Kinases	Dissociation time (hr)	• Dissociation time (nr)
HIVEP2 KLF4 FOSB OTX2 BHLHE40 MITF BHLHE41		SINAIT EGR3 SOX17 HIC1 LHX2 GTF2A2 OTX2 MEIS2	MAPK/ MAP2K1 PRKCD PRKCE Kinases EGF FGF2 AGT HGF VEGFA	CCL11 IL22 IL17A IL18 IL2 TNFSF14 IL4 IL7 MIF PF4 C5	FBXO32 CYP51A1 SIRT6 POR Enzymes mir-210 mir-26 mir-34	ERBB2 PRKCD SRC MAP3K14 EGFR RAF1 MAP2K1 FGFR1 PRKCE	Growth factors	niR-29b-3p miR-155-5p miR-296-5p let-7a-5p miR-30c-5p miR-124-3p miR-1-3p miR-16-5p		
SOX4 SOX10 HBP1 ING4 DDIT3 SATB1 CHURC1 SOX9 MXI1		THRA MITF PBX1 HAND2 SOX10 DMRTA1 NR2F2 MAF RAX DBP	AREG	Cytokines miR-17-5p miR-141-3p miR-26a-5p miR-26a-5p miR-26a-5p miR-293-5p miR-293-5p miR-293-5p miR-204-5p miR-193a-3p let-7a-5p	mir-145 mir-373 let-7 mir-146 mir-122 mir-126 mir-21 -3 0 3 Mature miRNA	NVAFNS IKBKB CHUK MAP3K1 MAP3K1 EIF2AK3 CDK9 PRKCB RET PRKCB PRKD1 JAK2 RIPK2 RIPK2 MAPK8	IL17A CSF2 OSM IL6 EDN1 IL3 CXCL12 TNFSF12 IL18 CSF1 SPP1 CCL5 IL5 CCL11	miRNA mir-515 mir-143 mir-34 mir-145 mir-145 mir-146 mir-122		
HLTF TEF TCF4 SOX5 MEIS1 PLAG1	log2(fpkm)	CEBPD CRX FOXJ1 HLF GSX2 log2(fpkm) 2 4 6	ANGPT2 GH1 BTC BTC HBEGF	miRNA		PRKCQ PRKCZ PRKCZ MAP2K4 LK FGFR3 PIK3CG DDR1 MKNK1 AKT1 TGFBR1 IPMK Kinases	TNFSF14 C5 IL1RN Cytokines	Mature miRNA		





D

Figure S4













## Figure S1. Transcriptomic profiling shows commonality between enzymatic dissociation and TGF- $\beta$ /TNF- $\alpha$ induced RPE EMT.

(A) Venn diagram represents the genes that shares the common number of genes between dissociation (3-48 hr) and TGF- $\beta$ /TNF- $\alpha$  (1-40 ng/mL) induced EMT-RPE. (B) Hierarchical clustering of log<sub>2</sub>-transformed ratios and average abundances of differentially expressed genes showing significant differences across time point after enzymatic dissociation and TGF- $\beta$ /TNF- $\alpha$  induced RPE-EMT. (C) Top canonical pathways were predicted based on the highly enriched genes that changed in abundance (activated or inhibited) during enzymatic dissociation and TGF- $\beta$ /TNF- $\alpha$  induced EMT were plotted based on p-value. (D-J) Heatmaps of IPA generated upstream regulator analysis from common genes that altered from both RPE-EMT. IPA uses activation Z-score as a statistical measure of the match between expected relationship direction and observed changes in gene expression regulator including (D) transcription regulators (E) kinases (F) enzymes(G) growth factors (H) cytokines (I-J) miRNAs.

# Figure S2. Top transcription factors and upstream regulators of enzymatic dissociation and TGF-β/TNF-α induced RPE-EMT.

Heat maps of top 20 transcription factors that up-regulated (EMT) and down-regulated (RPE) during enzymatic dissociation induced (A) and TGF- $\beta$ /TNF- $\alpha$  (B) induced RPE-EMT. (C) Heatmaps of IPA generated upstream regulators identified from dissociation (C) and TGF- $\beta$ /TNF- $\alpha$  (D) induced RPE-EMT. (E-F) Expression ratios for miRNA were plotted against log<sub>2</sub> fold change value from the RNA-seq analysis of dissociation induced RPE-EMT.

#### Figure S3. Correlation of RPE EMT gene expression ratios between RNA-seq and qRT PCR.

The expression ratios were plotted against log<sub>2</sub> fold change value from RNA-seq (x-axis) and qRT PCR was performed using the amplified cDNA from each RNAseq sample (y-axis). Statistically significant Pearson correlation is shown between the gene expression levels analyzed by qRT PCR and RNA-seq (A) Dissociation/Monolayer (39 genes), (B) TGF-B/TNF-A/Monolayer (54 genes). Principal Components Analysis (PCA) of gene expression data from dissociation (C) and TGF-

 $\beta$ /TNF- $\alpha$  treatment (D) were significantly correlated. Only genes with statistically significant changes at FDR of 5% (q<0.05) were included in analysis.

**Figure S4. Validation of dissociation induced RPE-EMT in hiPS (IMR90.4) RPE cells.** Differential expression of key EMT associated genes (A-C) and RPE specific genes (D) were measured by qRT PCR after enzymatic dissociation of monolayers (MLR) into single cells.

**Figure S5. Validation of TGF-** $\beta$ **/TNF-** $\alpha$  **induced RPE-EMT in hiPS (IMR90.4) RPE cells.** Differential expression of key EMT associated genes (A-B) and RPE specific genes (C) were measured by qRT PCR after enzymatic dissociation of monolayers (MLR) into single cells.

**Figure S6. Enzyme-free dissociation induced RPE-EMT.** Differential expression of EMT-associated factor due to the physical (using cell scraper) dissociation.

**Figure S7**. **Enzyme-free dissociation induced RPE-EMT.** Differential expression of RPE-associated factor due to the physical (using cell scraper) dissociation.

Dataset S01. List of genes that enriched during dissociation induced RPE-EMT

**Dataset S02.** List of genes that enriched during TGF- $\beta$ /TNF- $\alpha$  RPE EMT induced RPE-EMT

**Dataset S03.** List of genes that altered from both dissociation and TGF- $\beta$ /TNF- $\alpha$  induced RPE-EMT

Dataset S04. List of canonical pathways that enriched during dissociation induced RPE EMT

Dataset S05. List of canonical pathways that enriched during TGF-β/TNF-α RPE EMT

**Dataset S06.** List of canonical pathways that enriched in common from dissociation and TGF- $\beta$ /TNF- $\alpha$  induced RPE-EMT

Dataset S07. Upstream regulator analysis of enzymatic dissociation induced RPE EMT

**Dataset S08.** Upstream regulator analysis of TGF- $\beta$ /TNF- $\alpha$  induced RPE EMT **Dataset S09.** Upstream regulators that enriched in common from dissociation and TGF- $\beta$ /TNF- $\alpha$  induced RPE-EMT.