# Supplementary Information for
# Therapeutic MK2 Inhibition Blocks Pathological Vascular Smooth Muscle Cell Phenotype Switch.

J. William Tierney, Brian C. Evans, Joyce Cheung-Flynn, Bo Wang, Juan M. Colazo, Monica E. Polcz, Rebecca S. Cook, Colleen M. Brophy, Craig L. Duvall

Corresponding author: Craig Duvall
Email: craig.duvall@vanderbilt.edu

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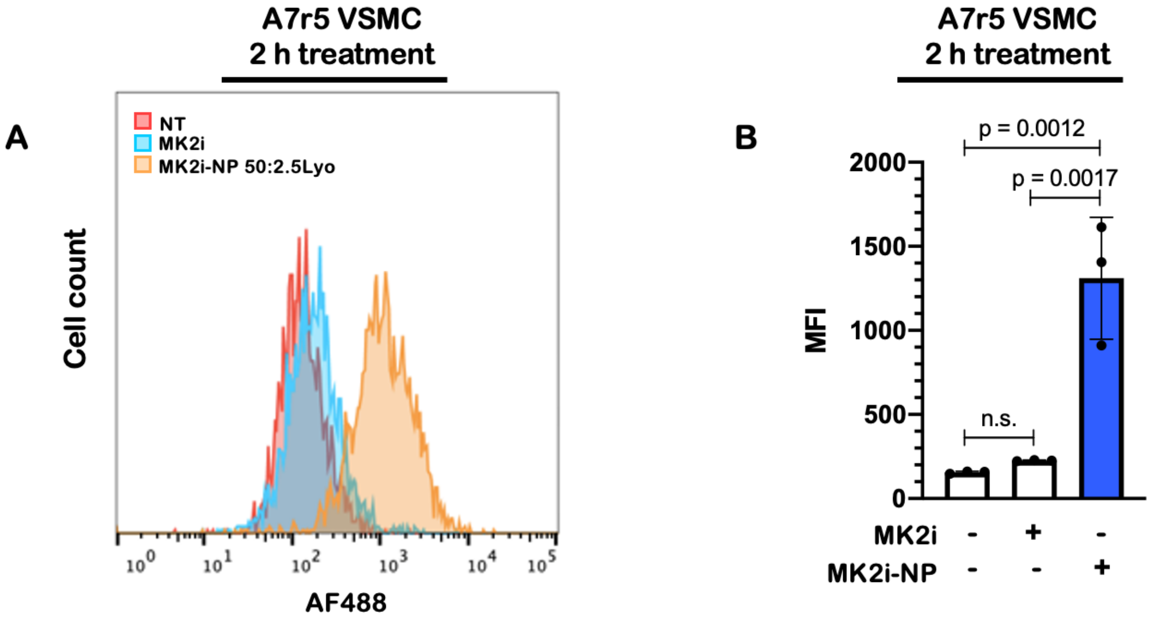


**Figure S1.** A7r5 cells were treated 2 hours with AlexFlour 488 (AF488)-labeled MK2i (50 μM), which was delivered as free-MK2i, or as MK2i-NP$^{50:2.5Lyo}$. Flow cytometric analysis was used to detect AF488 in cells. A. Representative histogram of flow cytometry experiments. B. Quantitation of the mean fluorescence intensity (MFI) per cell. Values shown are the average of (N = 3) experiments, ± S.D. P-values are derived from statistical analysis using one-way ANOVA with Tukey's repeated measures to compare individual groups.

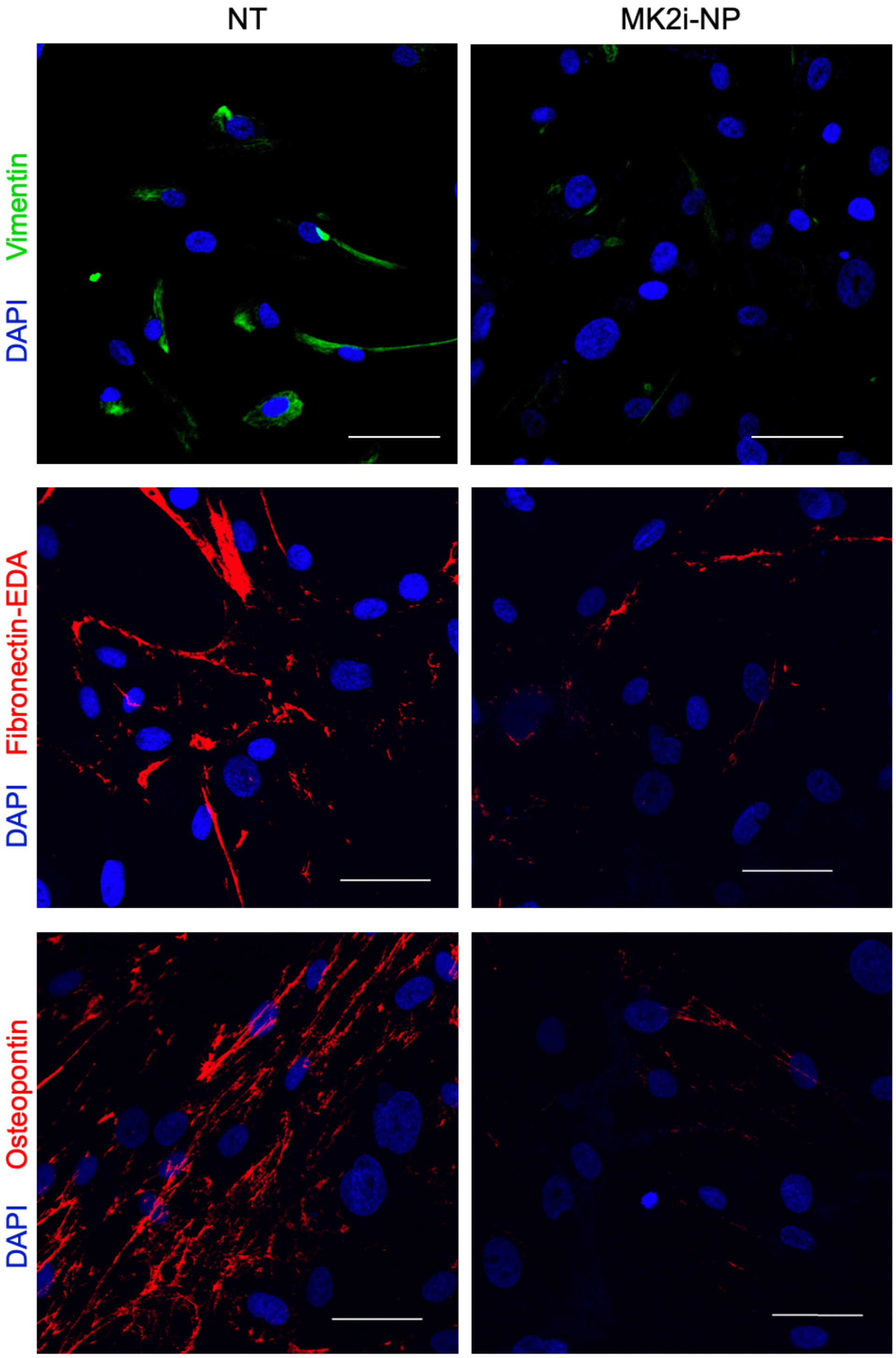


**Figure S2.** Immunocytochemistry shows reduction of multiple synthetic phenotype markers after MK2i-NP treatment. Primary human coronary artery smooth muscle cells were cultured out to P7 in 20% serum. Cells were either left untreated or treated with 50 μM MK2i-NPs for 2 hours at each passage. Scale bars are 50μm.

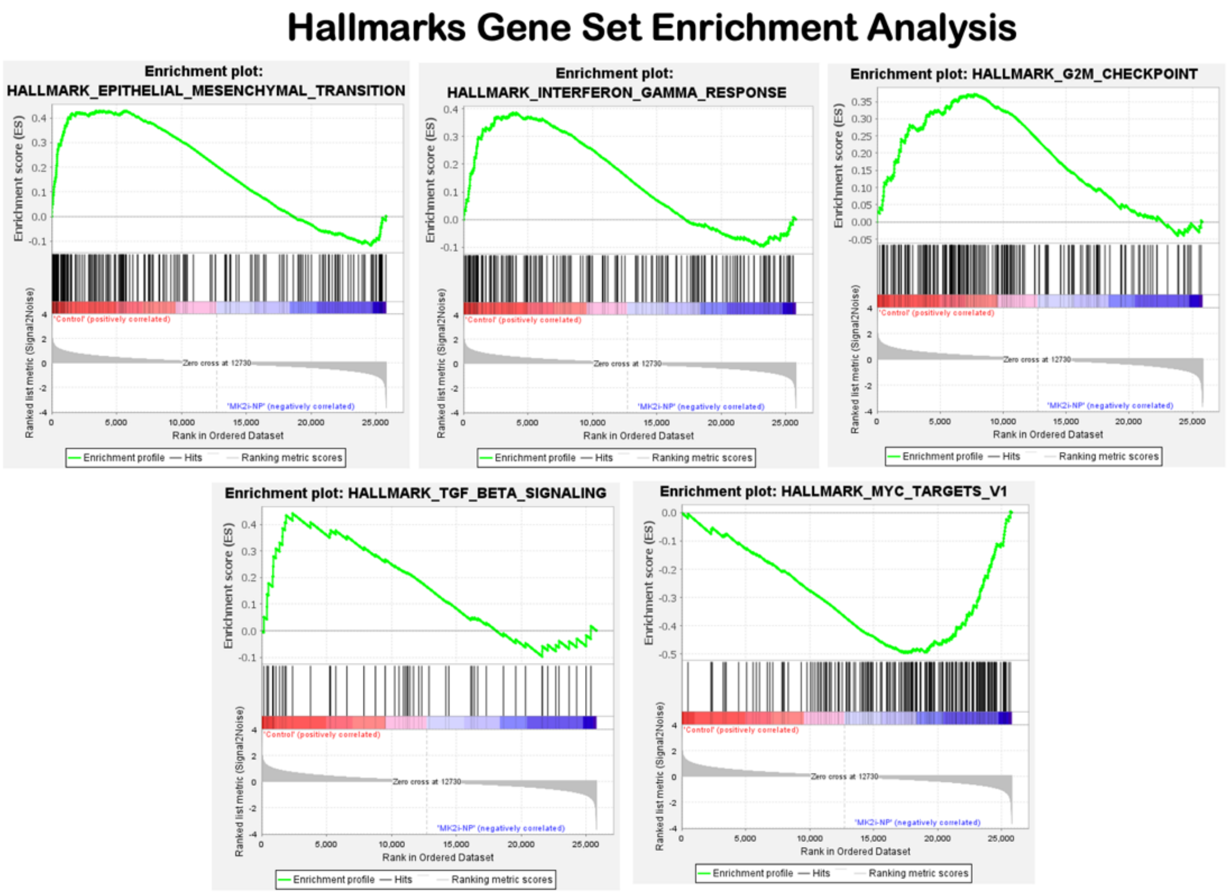


**Figure S3.** Significantly altered genes identified within RNA-Seq data of human primary coronary artery smooth muscle cells harvested from clinical samples treated with or without MK2i-NP were (N = 3 per group) were queried by Gene Set Enrichment Analysis using GSEA Software 4.1.0 (Broad Institute) for enrichment within the Hallmarks Gene Set (h.all.v7.3). Graphs were selected among the gene sets that were significantly correlated with altered genes in MK2i-NP-treated cells.

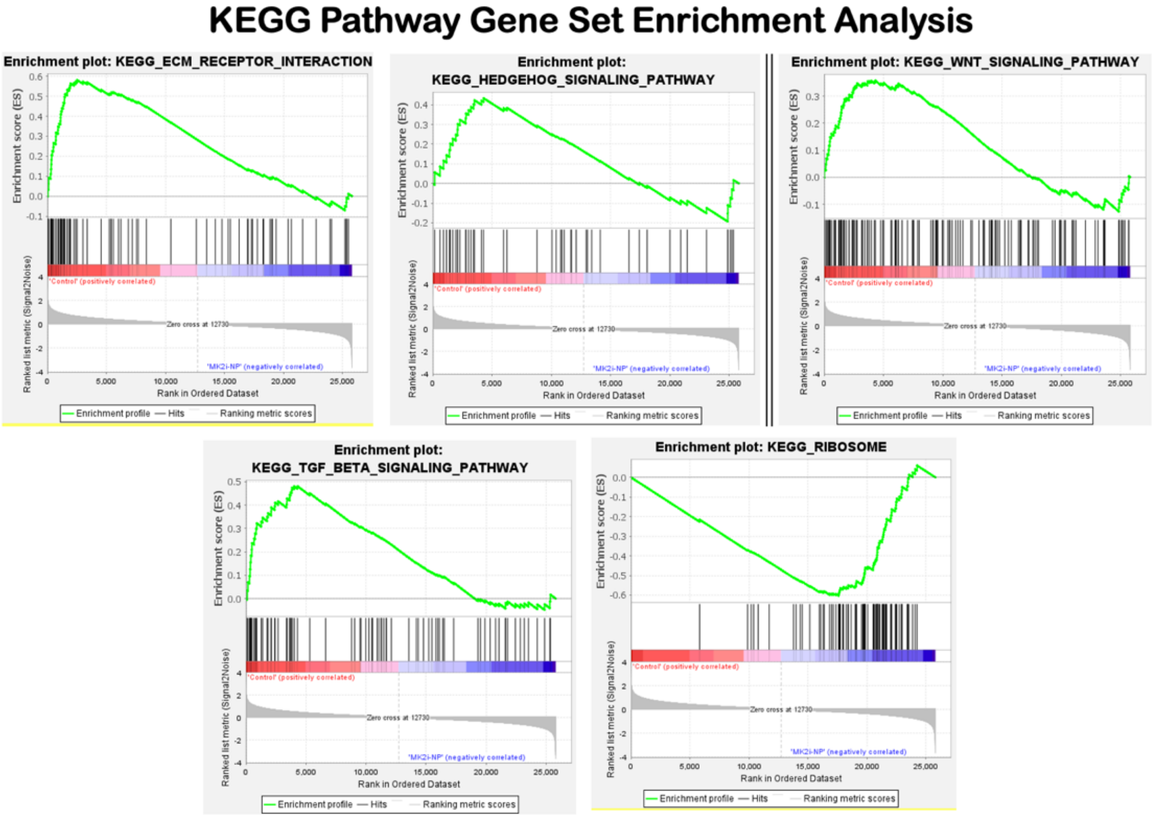


**Figure S4.** Significantly altered genes identified within RNA-Seq data of human primary coronary artery smooth muscle cells harvested from clinical samples treated with or without MK2i-NP were (N = 3 per group) were queried by Gene Set Enrichment Analysis using GSEA Software 4.1.0 (Broad Institute) for enrichment within the KEGG Pathway Gene Set (c2.cp.kegg.v7.1). Graphs were selected among the gene sets that were significantly correlated with altered genes in MK2i-NP-treated cells.

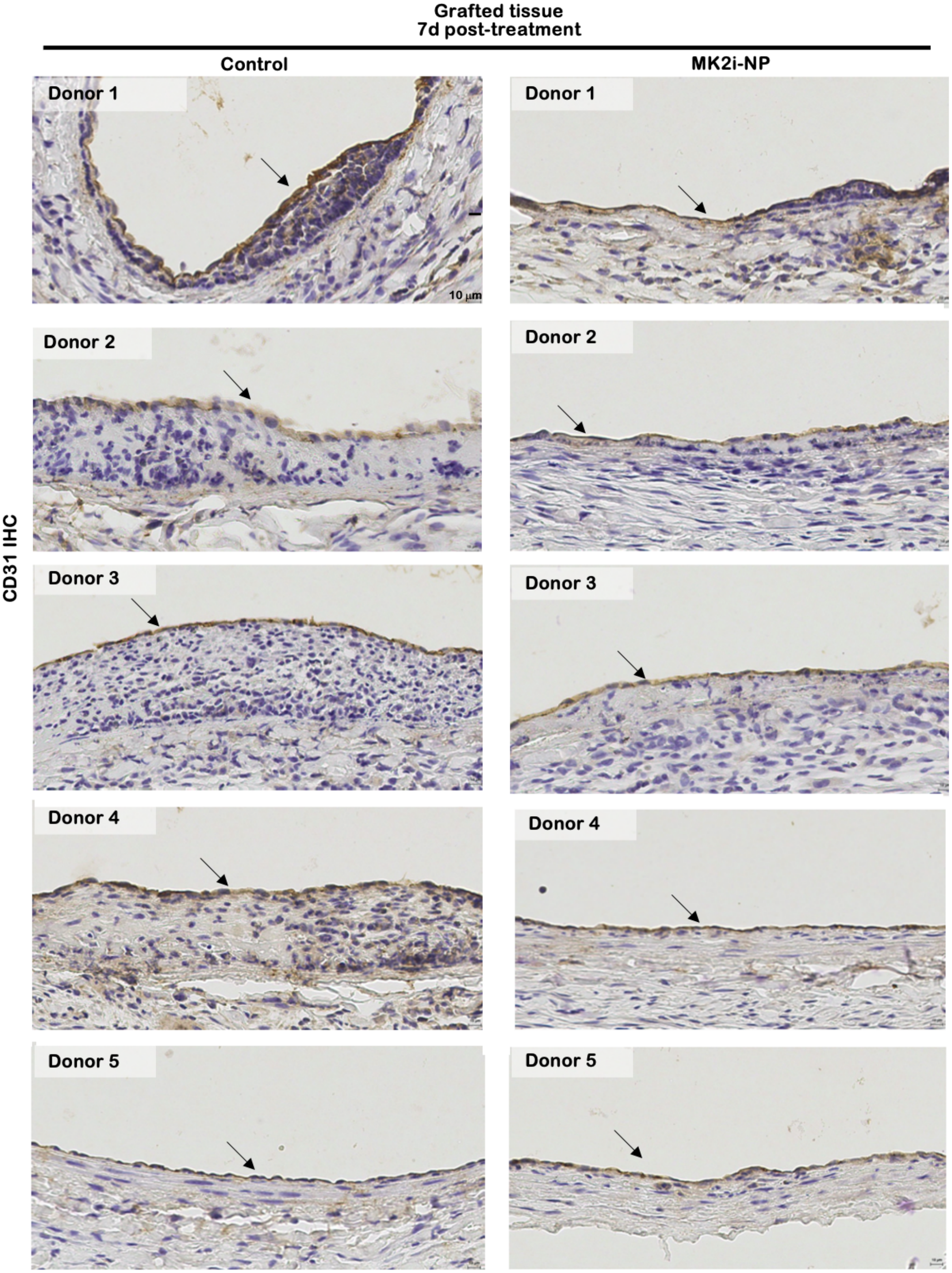
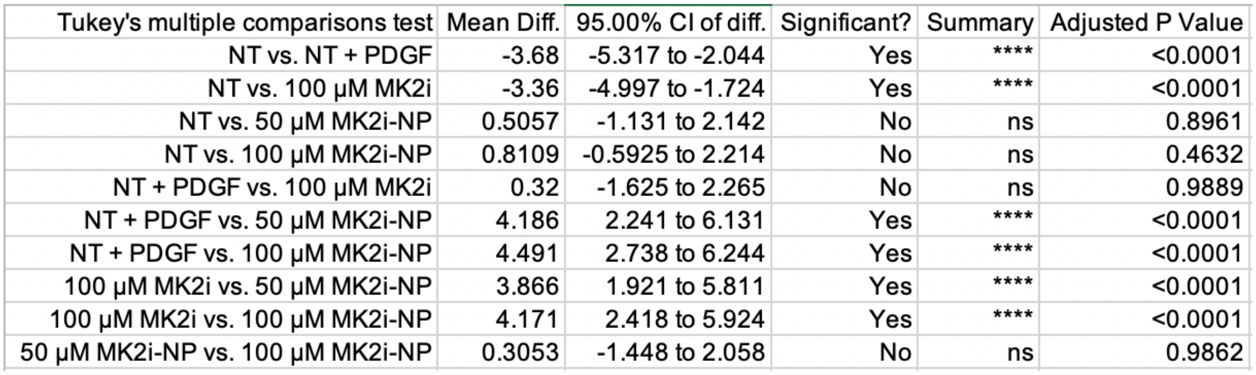


**Figure S5.** Rabbit vein grafts retain endothelial coverage after treatment and surgery. Rabbit external jugular veins were surgically excised, divided in half, treated 30 minutes with or without MK2i-NP$^{50:5Lyo}$, then surgically grafted onto contralateral carotid arteries of the same rabbit. After 7 days, grafted tissue was recovered and stained for CD31. Representative images from each grafted vein are shown.

**Table S1**. Statistical summary of ANOVA test done for 0.1% serum PDGF proliferation test.

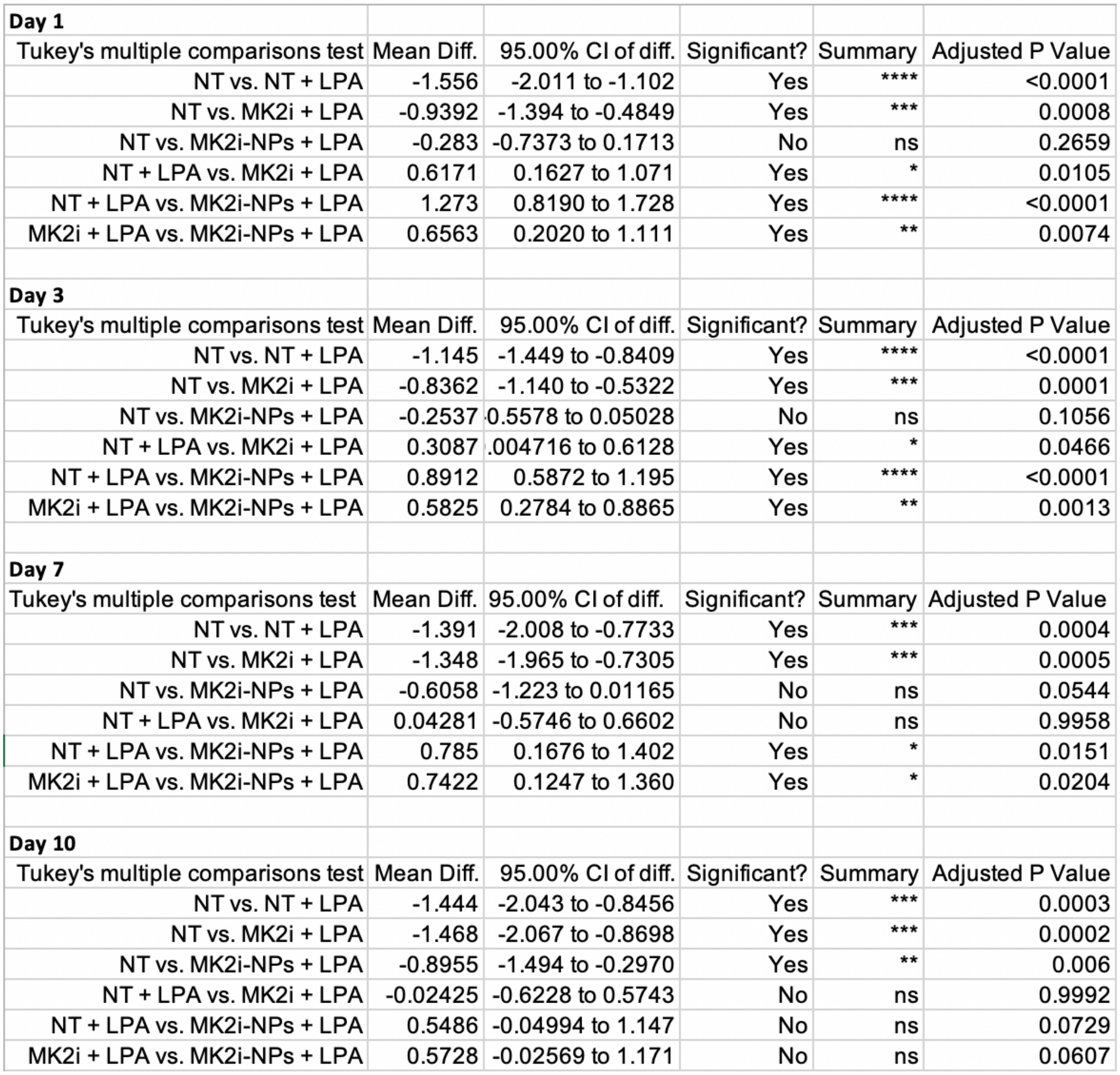


| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Significant? | Summary | Adjusted P Value |
|---:|---:|---:|---:|---:|---:|
| NT vs. NT + PDGF | -3.68 | -5.317 to -2.044 | Yes | **** | <0.0001 |
| NT vs. 100 μM MK2i | -3.36 | -4.997 to -1.724 | Yes | **** | <0.0001 |
| NT vs. 50 μM MK2i-NP | 0.5057 | -1.131 to 2.142 | No | ns | 0.8961 |
| NT vs. 100 μM MK2i-NP | 0.8109 | -0.5925 to 2.214 | No | ns | 0.4632 |
| NT + PDGF vs. 100 μM MK2i | 0.32 | -1.625 to 2.265 | No | ns | 0.9889 |
| NT + PDGF vs. 50 μM MK2i-NP | 4.186 | 2.241 to 6.131 | Yes | **** | <0.0001 |
| NT + PDGF vs. 100 μM MK2i-NP | 4.491 | 2.738 to 6.244 | Yes | **** | <0.0001 |
| 100 μM MK2i vs. 50 μM MK2i-NP | 3.866 | 1.921 to 5.811 | Yes | **** | <0.0001 |
| 100 μM MK2i vs. 100 μM MK2i-NP | 4.171 | 2.418 to 5.924 | Yes | **** | <0.0001 |
| 50 μM MK2i-NP vs. 100 μM MK2i-NP | 0.3053 | -1.448 to 2.058 | No | ns | 0.9862 |

**Table S2.** Statistical summary of ANOVA test done for A7r5 VSMC viability experiment.

| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Significant? | Summary | Adjusted P Value |
|---|---|---|---|---|---|
| NT vs. MK2i | 0.01946 | -0.04338 to 0.08230 | No | ns | 0.8415 |
| NT vs. MK2i-NPs | 0.05197 | -0.01087 to 0.1148 | No | ns | 0.1369 |
| NT vs. Pos. Cont. | 0.9735 | 0.9106 to 1.036 | Yes | **** | <0.0001 |
| MK2i vs. MK2i-NPs | 0.03252 | -0.03032 to 0.09536 | No | ns | 0.5174 |
| MK2i vs. Pos. Cont. | 0.954 | 0.8912 to 1.017 | Yes | **** | <0.0001 |
| MK2i-NPs vs. Pos. Cont. | 0.9215 | 0.8587 to 0.9844 | Yes | **** | <0.0001 |

**Table S3.** Full one-way ANOVA results for CREB phosphorylation western blots on days 1, 3, 7, and 10.



| | | | | | |
|---|---|---|---|---|---|
| **Day 1** | | | | | |
| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Significant? | Summary | Adjusted P Value |
| NT vs. NT + LPA | -1.556 | -2.011 to -1.102 | Yes | **** | <0.0001 |
| NT vs. MK2i + LPA | -0.9392 | -1.394 to -0.4849 | Yes | *** | 0.0008 |
| NT vs. MK2i-NPs + LPA | -0.283 | -0.7373 to 0.1713 | No | ns | 0.2659 |
| NT + LPA vs. MK2i + LPA | 0.6171 | 0.1627 to 1.071 | Yes | * | 0.0105 |
| NT + LPA vs. MK2i-NPs + LPA | 1.273 | 0.8190 to 1.728 | Yes | **** | <0.0001 |
| MK2i + LPA vs. MK2i-NPs + LPA | 0.6563 | 0.2020 to 1.111 | Yes | ** | 0.0074 |
| | | | | | |
| **Day 3** | | | | | |
| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Significant? | Summary | Adjusted P Value |
| NT vs. NT + LPA | -1.145 | -1.449 to -0.8409 | Yes | **** | <0.0001 |
| NT vs. MK2i + LPA | -0.8362 | -1.140 to -0.5322 | Yes | *** | 0.0001 |
| NT vs. MK2i-NPs + LPA | -0.2537 | -0.5578 to 0.05028 | No | ns | 0.1056 |
| NT + LPA vs. MK2i + LPA | 0.3087 | .004716 to 0.6128 | Yes | * | 0.0466 |
| NT + LPA vs. MK2i-NPs + LPA | 0.8912 | 0.5872 to 1.195 | Yes | **** | <0.0001 |
| MK2i + LPA vs. MK2i-NPs + LPA | 0.5825 | 0.2784 to 0.8865 | Yes | ** | 0.0013 |
| | | | | | |
| **Day 7** | | | | | |
| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Significant? | Summary | Adjusted P Value |
| NT vs. NT + LPA | -1.391 | -2.008 to -0.7733 | Yes | *** | 0.0004 |
| NT vs. MK2i + LPA | -1.348 | -1.965 to -0.7305 | Yes | *** | 0.0005 |
| NT vs. MK2i-NPs + LPA | -0.6058 | -1.223 to 0.01165 | No | ns | 0.0544 |
| NT + LPA vs. MK2i + LPA | 0.04281 | -0.5746 to 0.6602 | No | ns | 0.9958 |
| NT + LPA vs. MK2i-NPs + LPA | 0.785 | 0.1676 to 1.402 | Yes | * | 0.0151 |
| MK2i + LPA vs. MK2i-NPs + LPA | 0.7422 | 0.1247 to 1.360 | Yes | * | 0.0204 |
| | | | | | |
| **Day 10** | | | | | |
| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Significant? | Summary | Adjusted P Value |
| NT vs. NT + LPA | -1.444 | -2.043 to -0.8456 | Yes | *** | 0.0003 |
| NT vs. MK2i + LPA | -1.468 | -2.067 to -0.8698 | Yes | *** | 0.0002 |
| NT vs. MK2i-NPs + LPA | -0.8955 | -1.494 to -0.2970 | Yes | ** | 0.006 |
| NT + LPA vs. MK2i + LPA | -0.02425 | -0.6228 to 0.5743 | No | ns | 0.9992 |
| NT + LPA vs. MK2i-NPs + LPA | 0.5486 | -0.04994 to 1.147 | No | ns | 0.0729 |
| MK2i + LPA vs. MK2i-NPs + LPA | 0.5728 | -0.02569 to 1.171 | No | ns | 0.0607 |

**Table S4.** List of genes in KEGG_TGF_BETA_SIGNALING_PATHWAY, which was used to query RNA-Seq data of human primary coronary artery smooth muscle cells harvested from clinical samples treated with or without MK2i-NP were (N = 3 per group). An enrichment score for each curated gene for this KEGG Pathway was calculated using GSEA 4.1.0 software. The associated fold change for each gene is shown, as determined by RNA-Seq analysis, comparing expression changes in cells treated with MK2i-NP versus untreated cells.

| GENE SYMBOL | Enrichment Score | Log2 Fold Change (MK2i-NP versus Control) | P-Value |
|---|---|---|---|
| THBS2 | 0.0352 | -1.4651917 | 8.01E-08 |
| BMP6 | 0.0699 | -1.569711 | 1.93E-06 |
| CDKN2B | 0.0972 | -1.1399918 | 0.00282506 |
| LTBP1 | 0.1263 | -1.0227961 | 8.50E-05 |
| RBL2 | 0.1558 | -0.8357188 | 0.00242486 |
| SMAD3 | 0.1843 | -0.8597901 | 0.00168497 |
| INHBE | 0.2101 | -1.0714435 | 0.07986732 |
| MYC | 0.238 | -0.7914765 | 0.00214905 |
| SP1 | 0.2574 | -0.6287946 | 0.00181864 |
| TGFB3 | 0.2797 | -2.4841598 | 5.71E-07 |
| SMAD1 | 0.3007 | -1.0901315 | 0.00191102 |
| BMPR2 | 0.3223 | -0.572002 | 0.01008074 |
| TGFB2 | 0.3297 | -1.5769365 | 0.00020903 |
| TGFBR1 | 0.3463 | -0.6129673 | 0.03114465 |
| CHRD | 0.3506 | -0.9996664 | 0.04378031 |
| COMP | 0.3659 | n.d. | n.d. |
| SMAD4 | 0.3731 | -0.4125166 | 0.05024536 |
| BMP8A | 0.3903 | n.s. | n.s. |
| THBS3 | 0.3959 | n.s. | n.s. |
| BMPR1B | 0.4109 | n.s. | n.s. |
| SMAD5 | 0.4166 | n.s. | n.s. |
| DCN | 0.4031 | n.s. | n.s. |
| ID4 | 0.415 | n.s. | n.s. |
| CREBBP | 0.4283 | n.s. | n.s. |
| ROCK1 | 0.4324 | n.s. | n.s. |
| EP300 | 0.4448 | n.s. | n.s. |
| ZFYVE9 | 0.4546 | n.s. | n.s. |
| ROCK2 | 0.464 | n.s. | n.s. |
| RPS6KB1 | 0.4752 | n.s. | n.s. |
| ZFYVE16 | 0.4806 | n.s. | n.s. |
| ACVR2A | 0.4812 | n.s. | n.s. |
| BMPR1A | 0.4509 | n.s. | n.s. |
| E2F5 | 0.4067 | n.s. | n.s. |
| BMP4 | 0.3275 | n.s. | n.s. |
| GDF6 | 0.3219 | n.s. | n.s. |
| ID3 | 0.3074 | n.s. | n.s. |
| TGFBR2 | 0.3082 | n.s. | n.s. |
| SMAD9 | 0.2955 | n.s. | n.s. |
| ID2 | 0.2877 | n.s. | n.s. |
| ACVR1C | 0.2805 | n.s. | n.s. |

| GENE SYMBOL | Enrichment Score | Log2 Fold Change (MK2i-NP versus Control) | P-Value |
|---|---|---|---|
| ACVR2B | 0.2818 | n.s. | n.s. |
| GDF7 | 0.2828 | n.s. | n.s. |
| TGFB1 | 0.2696 | n.s. | n.s. |
| ACVR1 | 0.2654 | n.s. | n.s. |
| NODAL | 0.2588 | n.s. | n.s. |
| RHOA | 0.26 | n.s. | n.s. |
| SMAD2 | 0.2535 | n.s. | n.s. |
| PITX2 | 0.2461 | n.s. | n.s. |
| TFDP1 | 0.2464 | n.s. | n.s. |
| SMAD6 | 0.231 | n.s. | n.s. |
| RBL1 | 0.1752 | n.s. | n.s. |
| SMURF1 | 0.1526 | n.s. | n.s. |
| FST | 0.1484 | n.s. | n.s. |
| RBX1 | 0.1342 | n.s. | n.s. |
| CUL1 | 0.1196 | n.s. | n.s. |
| THBS4 | 0.1124 | n.s. | n.s. |
| MAPK1 | 0.1036 | n.s. | n.s. |
| GDF5 | 0.101 | n.s. | n.s. |
| SMAD7 | 0.1001 | n.s. | n.s. |
| ACVRL1 | 0.0935 | n.s. | n.s. |
| PPP2R1B | 0.0934 | n.s. | n.s. |
| SMURF2 | 0.0679 | n.s. | n.s. |
| E2F4 | -0.0024 | n.s. | n.s. |
| RPS6KB2 | -0.0023 | n.s. | n.s. |
| PPP2R1A | -0.0076 | n.s. | n.s. |
| PPP2CA | -0.0147 | n.s. | n.s. |
| INHBB | -0.0215 | n.s. | n.s. |
| PPP2CB | -0.0214 | n.s. | n.s. |
| ID1 | -0.0303 | n.s. | n.s. |
| THBS1 | -0.0183 | n.s. | n.s. |
| INHBC | -0.0123 | n.s. | n.s. |
| INHBA | -0.0229 | n.s. | n.s. |
| SKP1 | -0.024 | n.s. | n.s. |
| MAPK3 | -0.0205 | 0.37510553 | 0.0710939 |
| TNF | -0.0168 | n.s. | n.s. |
| NOG | -0.0247 | n.s. | n.s. |
| BMP8B | -0.0239 | n.s. | n.s. |
| AMH | -0.0115 | n.s. | n.s. |
| BMP2 | 0.0173 | 1.05002194 | 0.0009175 |

**Table S5.** List of genes in KEGG_ECM_RECEPTOR_INTERACTION, which was used to query RNA-Seq data of human primary coronary artery smooth muscle cells harvested from clinical samples treated with or without MK2i-NP were (N = 3 per group). An enrichment score for each curated gene for this KEGG Pathway was calculated using GSEA 4.1.0 software. The associated fold change for each gene is shown, as determined by RNA-Seq analysis, comparing expression changes in cells treated with MK2i-NP versus untreated cells.

| Gene Symbol | Enrichment Score | Log2 Fold Change (MK2i-NP versus Control) | P-Value |
|---|---|---|---|
| LAMA5 | 1.852 | -2.083186249 | 1.44E-12 |
| COL5A2 | 1.621 | -1.688775328 | 6.26E-08 |
| THBS2 | 1.597 | -1.46519165 | 8.01E-08 |
| COL1A1 | 1.334 | -1.612901168 | 8.70E-07 |
| COL4A1 | 1.293 | -1.00138327 | 0.000388 |
| COL5A1 | 1.276 | -1.66205986 | 1.60E-06 |
| HSPG2 | 1.265 | -1.462198497 | 7.16E-05 |
| LAMA2 | 1.247 | -1.437068621 | 5.84E-05 |
| COL1A2 | 1.203 | -1.391982992 | 7.56E-06 |
| COL4A2 | 1.145 | -0.895038811 | 0.00148 |
| COL5A3 | 1.139 | -2.547953268 | 0.002021 |
| LAMB2 | 1.051 | -0.759354914 | 0.003482 |
| LAMC1 | 1.042 | n.s. | n.s. |
| LAMB1 | 1.041 | -1.030755447 | 0.000752 |
| COL3A1 | 0.975 | -2.387621616 | 4.24E-06 |
| ITGAV | 0.973 | -0.629867351 | 0.014756 |
| FN1 | 0.944 | -1.044944594 | 0.020816 |
| ITGA4 | 0.914 | -0.604567027 | 0.036033 |
| COL4A4 | 0.91 | -1.841605114 | 0.001085 |
| ITGA1 | 0.908 | -1.134842949 | 0.003289 |
| COL4A6 | 0.897 | -1.135307151 | 0.003179 |
| COL6A3 | 0.88 | -1.170899677 | 0.001265 |
| ITGA8 | 0.855 | | |
| AGRN | 0.85 | -0.847987373 | 0.007853 |
| COL6A1 | 0.831 | -0.805428447 | 0.009089 |
| COL6A2 | 0.831 | -0.834331833 | 0.008532 |
| VWF | 0.82 | -2.964830426 | 0.000679 |
| LAMA4 | 0.779 | -0.740264755 | 0.017725 |
| ITGA10 | 0.779 | -0.789340112 | 0.045694 |
| COMP | 0.746 | n.d. | n.d. |
| VTN | 0.736 | n.s. | n.s. |
| ITGB8 | 0.727 | -1.772523292 | 0.001398 |
| ITGA9 | 0.681 | n.s. | n.s. |
| THBS3 | 0.664 | n.s. | n.s. |
| COL6A6 | 0.648 | n.s. | n.s. |
| ITGA6 | 0.588 | n.s. | n.s. |
| ITGB1 | 0.547 | n.s. | n.s. |
| DAG1 | 0.442 | n.s. | n.s. |
| ITGA2B | 0.387 | n.s. | n.s. |
| ITGA11 | 0.376 | n.s. | n.s. |
| HMMR | 0.373 | n.s. | n.s. |
| TNXB | 0.362 | n.s. | n.s. |
| SV2A | 0.332 | n.s. | n.s. |
| CD47 | 0.318 | n.s. | n.s. |
| SDC3 | 0.282 | n.s. | n.s. |
| TNC | 0.264 | n.s. | n.s. |
| LAMA3 | 0.241 | n.s. | n.s. |
| SDC2 | 0.234 | n.s. | n.s. |
| TNR | 0.201 | n.s. | n.s. |
| LAMC2 | 0.088 | n.s. | n.s. |
| ITGB5 | 0.001 | n.s. | n.s. |
| COL11A2 | -0.033 | n.s. | n.s. |
| ITGA5 | -0.069 | n.s. | n.s. |
| LAMB3 | -0.095 | n.s. | n.s. |
| GP6 | -0.12 | n.s. | n.s. |
| THBS4 | -0.13 | n.s. | n.s. |
| ITGB6 | -0.166 | n.s. | n.s. |
| COL2A1 | -0.198 | n.s. | n.s. |
| ITGA7 | -0.2 | n.s. | n.s. |
| ITGA3 | -0.213 | n.s. | n.s. |
| GP5 | -0.219 | n.s. | n.s. |
| CHAD | -0.243 | n.s. | n.s. |
| CD36 | -0.267 | n.s. | n.s. |
| LAMC3 | -0.27 | n.s. | n.s. |
| RELN | -0.299 | n.s. | n.s. |
| LAMA1 | -0.308 | n.s. | n.s. |
| COL11A1 | -0.313 | n.s. | n.s. |
| ITGB4 | -0.332 | n.s. | n.s. |
| LAMB4 | -0.398 | n.s. | n.s. |
| ITGA2 | -0.419 | n.s. | n.s. |
| THBS1 | -0.493 | n.s. | n.s. |
| GP1BA | -0.609 | n.s. | n.s. |
| SDC4 | -0.763 | n.s. | n.s. |
| ITGB3 | -0.786 | 0.954969832 | 0.006175 |
| ITGB7 | -1.128 | n.s. | n.s. |
| SDC1 | -1.205 | 0.704474092 | 0.003958 |
| SPP1 | -1.216 | 4.902028593 | 3.93E-05 |
| CD44 | -1.344 | 0.790959797 | 4.59E-05 |