# A functional siRNA screen identifies genes modulating angiotensin II-mediated EGFR transactivation

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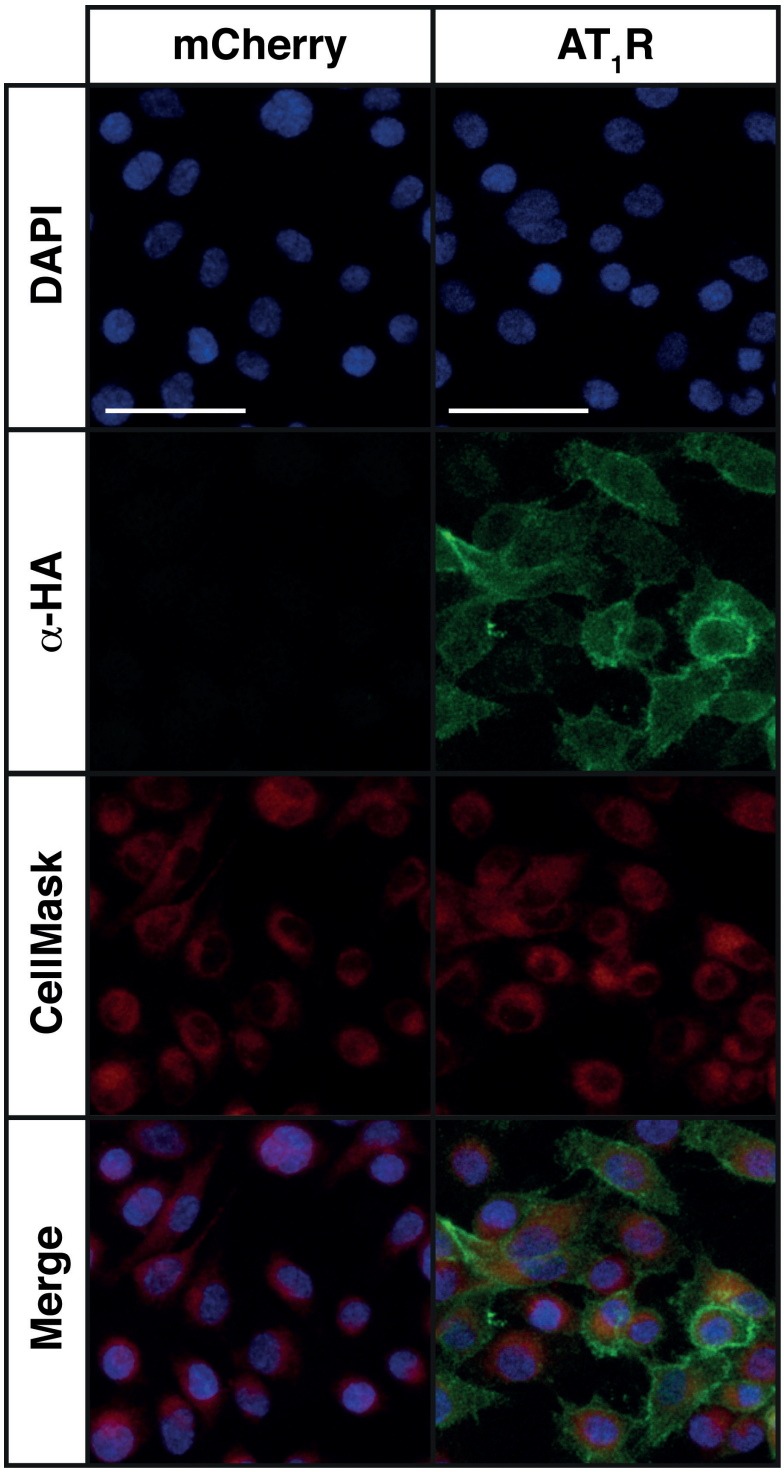
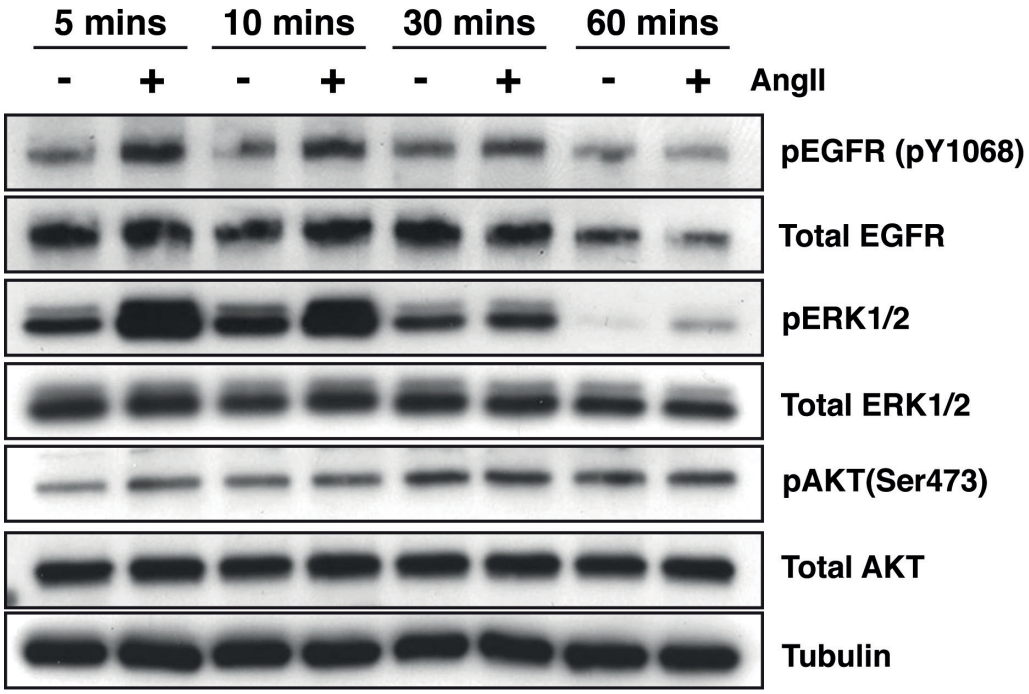
[1] School of Biomedical Sciences, The University of Queensland, St. Lucia, Queensland, 4072, Australia; [2] Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, Victoria, 3010, Australia; [3] Oncogenic Signalling and Growth Control Program, Peter MacCallum Cancer Centre, East Melbourne, Victoria, 3002, Australia; [4] The Victorian Centre for Functional Genomics, Peter MacCallum Cancer, East Melbourne, Victoria, 3002, Australia; [5] Department of Pathology, The University of Melbourne, Parkville, Victoria, 3010, Australia; [6] Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, 3004, Australia; [7] Department of Biochemistry and Molecular Biology, The University of Melbourne, Parkville, Victoria, 3010, Australia; [8] Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, 3800, Australia.

## Supplementary Figures

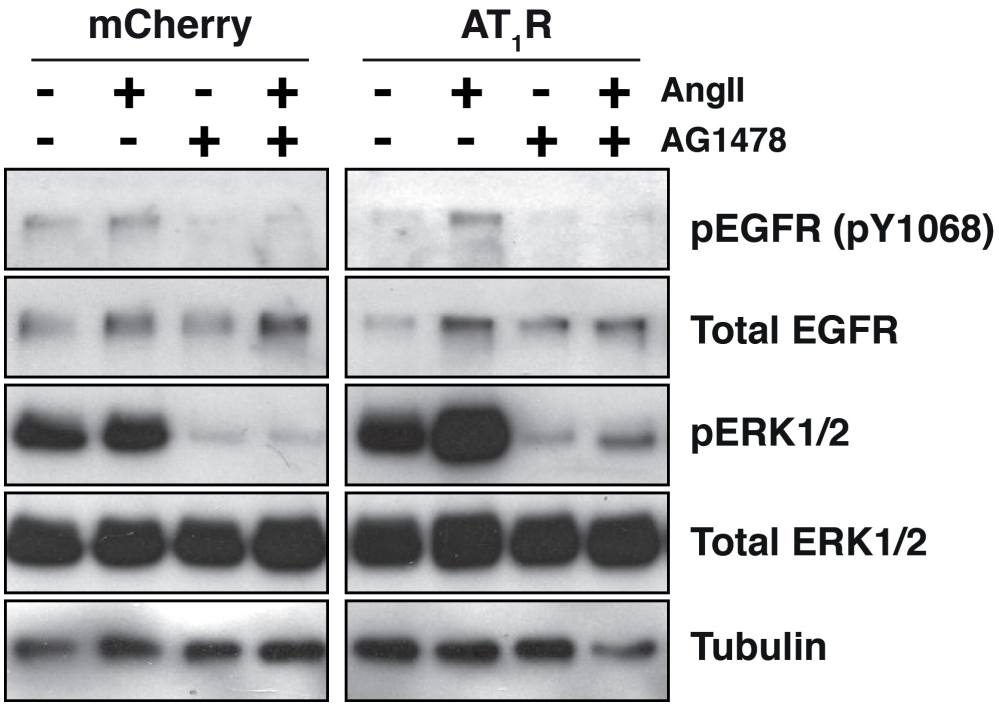
### Contains:



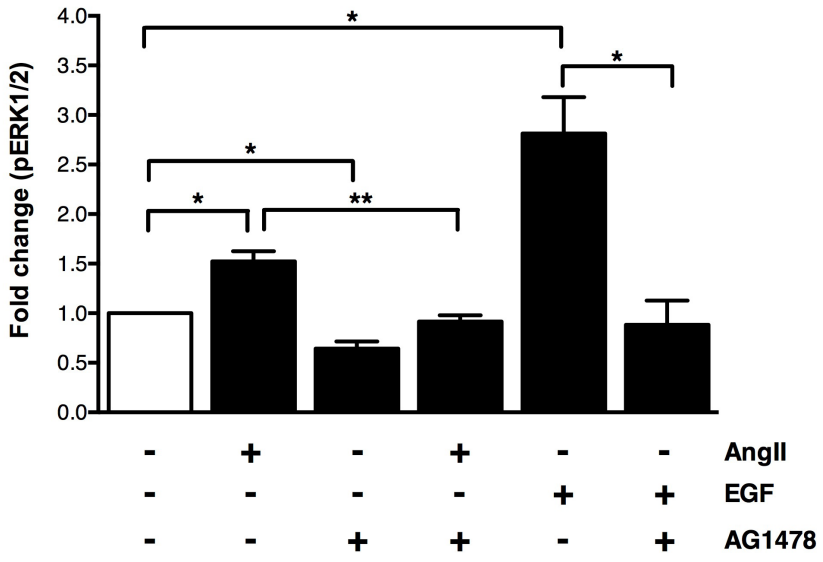

**Supplementary Figure 1: Immunofluorescence to detect $AT_1R$ receptor expression (individual and merged confocal images).** HMEC-LST-mCherry (mCherry) or HMEC-LST-$AT_1R$ ($AT_1R$) cells were stained with an anti-HA antibody (α-HA) to detect the ectopically expressed $AT_1R$, counterstained with DAPI to detect the nuclei, and CellMask to stain the whole cell. Confocal images were taken as described in Materials and Methods. Scale bars represent 50μm. The DAPI and α-HA channel merge for each cell line is displayed in Figure 1C. Confocal analysis was carried out on n=3 independent experiments; data is representative of all experiments performed.



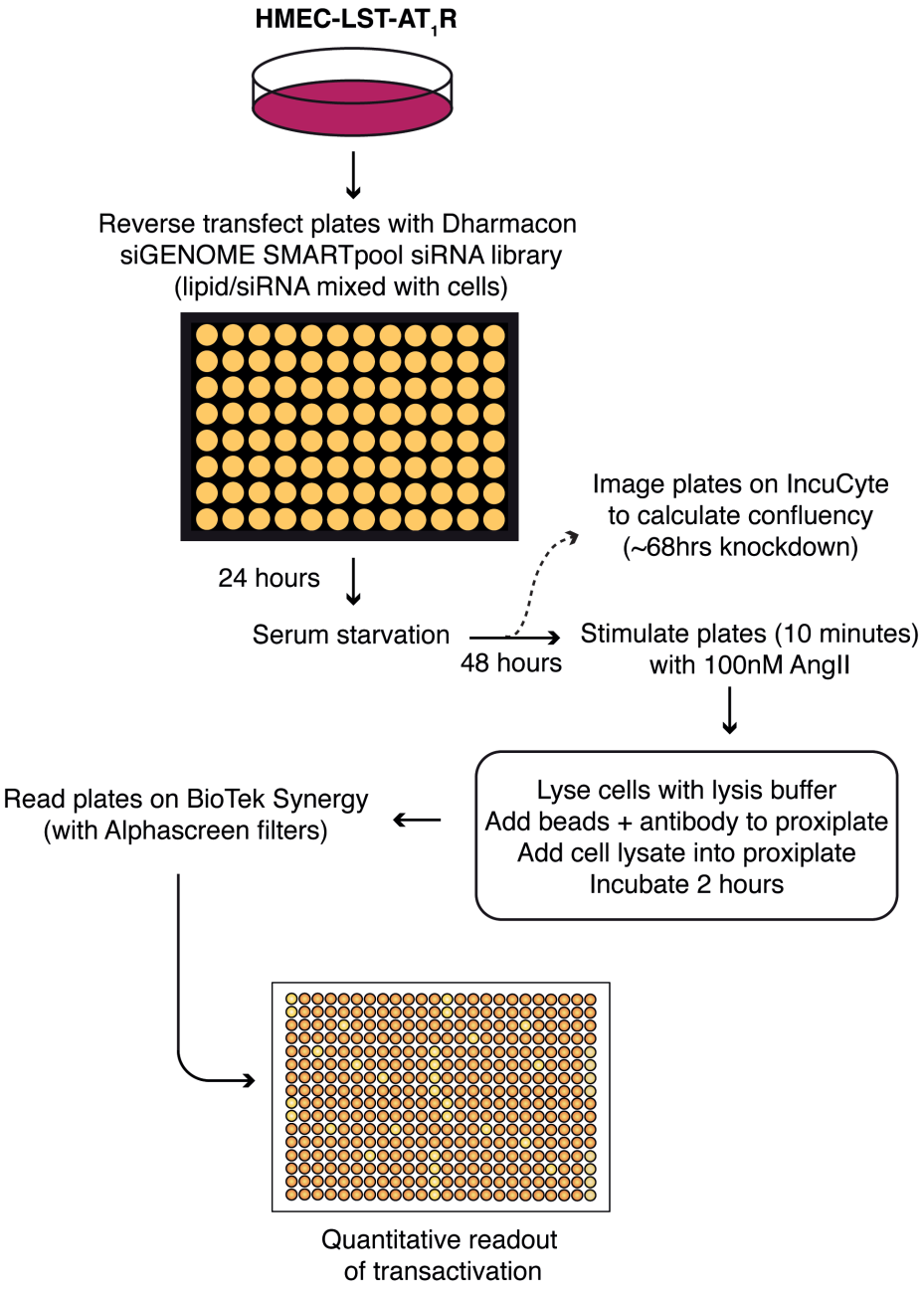

**Supplementary Figure 2: AngII stimulation timecourse analysis for the HMEC-LST-AT$_1$R cells.** HMEC-LST-AT$_1$R cells were stimulated with 100 nM AngII for the indicated times to determine the optimal activation of EGFR and ERK1/2 (as described in the Materials and Methods section). The involvement of the PI3K/AKT signaling pathway after AngII stimulation was also investigated using the phosphorylation of AKT (at the serine 473 phosphorylation site) as a functional readout of the pathway. Data presented is a representative of n=3 independent experiments.



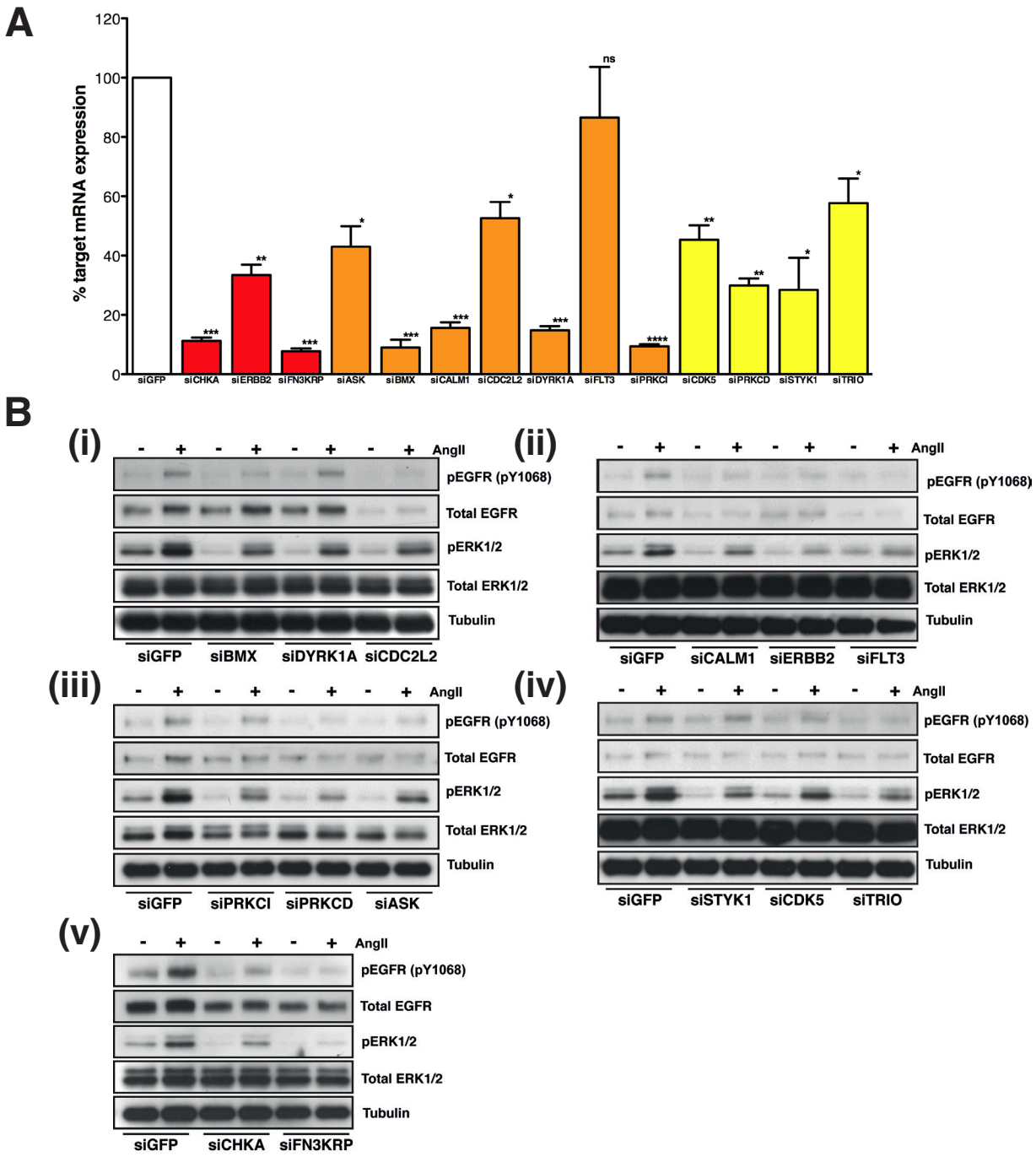

**Supplementary Figure 3: $AT_1R$-EGFR transactivation occurs in HMEC-HMLE cells.** HMEC-HMLE cells were transduced with the mCherry or $AT_1R$ constructs (as described in Materials and Methods). The cells were serum starved for 24 hours, pretreated with 5 μM AG1478 for 30 minutes, then stimulated with 100 nM AngII for 5 minutes and harvested (as described in the Materials and Methods section), to determine the level of $AT_1R$-EGFR transactivation (pEGFR and pERK1/2). Data is a representative of n=2 experiments.



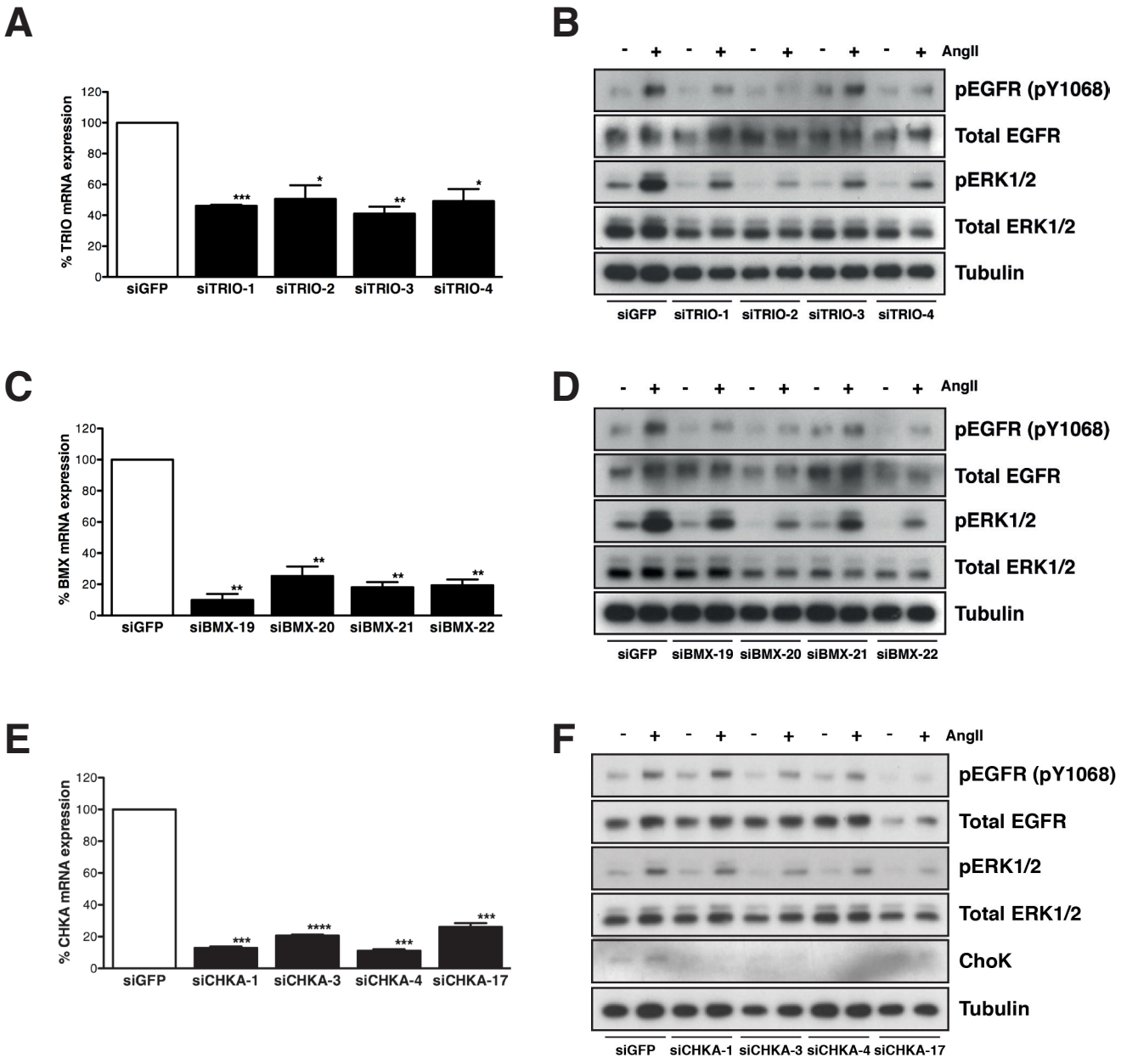

**Supplementary Figure 4: Measurement of $AT_1R$-EGFR transactivation in HMEC-LST-$AT_1R$ cells using the AlphaScreen SureFire phospho-ERK1/2 assay.** An $AT_1R$-EGFR transactivation assay, developed for 96 well microplate format using the HMEC-LST-$AT_1R$ cells and utilising the AlphaScreen SureFire phospho-ERK1/2 assay was used to quantify the phosphorylation of ERK1/2 after stimulation of cells with 100 nM AngII or 100 ng/ml EGF for 10 minutes in the presence or absence of 5 μM AG1748 (pretreated for 30 minutes prior to ligand stimulation). Data for each individual well was normalised to the calculated IncuCyte confluency. In each independent assay, n=8 wells for each condition were assayed; n=3 independent experiments were performed. Statistics: paired Student 2-tailed t-test, * $p < 0.05$, ** $p < 0.01$. Individual *p* values: unstimulated vs AngII stimulated, $p$=0.0358; unstimulated vs AG1478, $p$=0.0402; unstimulated vs EGF stimulated, $p$=0.0388; AngII stimulated vs AngII + AG1478, $p$=0.0049; EGF stimulated vs EGF + AG1478, $p$=0.0120. Note: the optimisation assay was performed as similarly described in the MIARE in the Supplementary siRNA Screen Data file, except that the cells were plated at a density of 9000 cells/well (without transfection) in a final volume of 100μl.



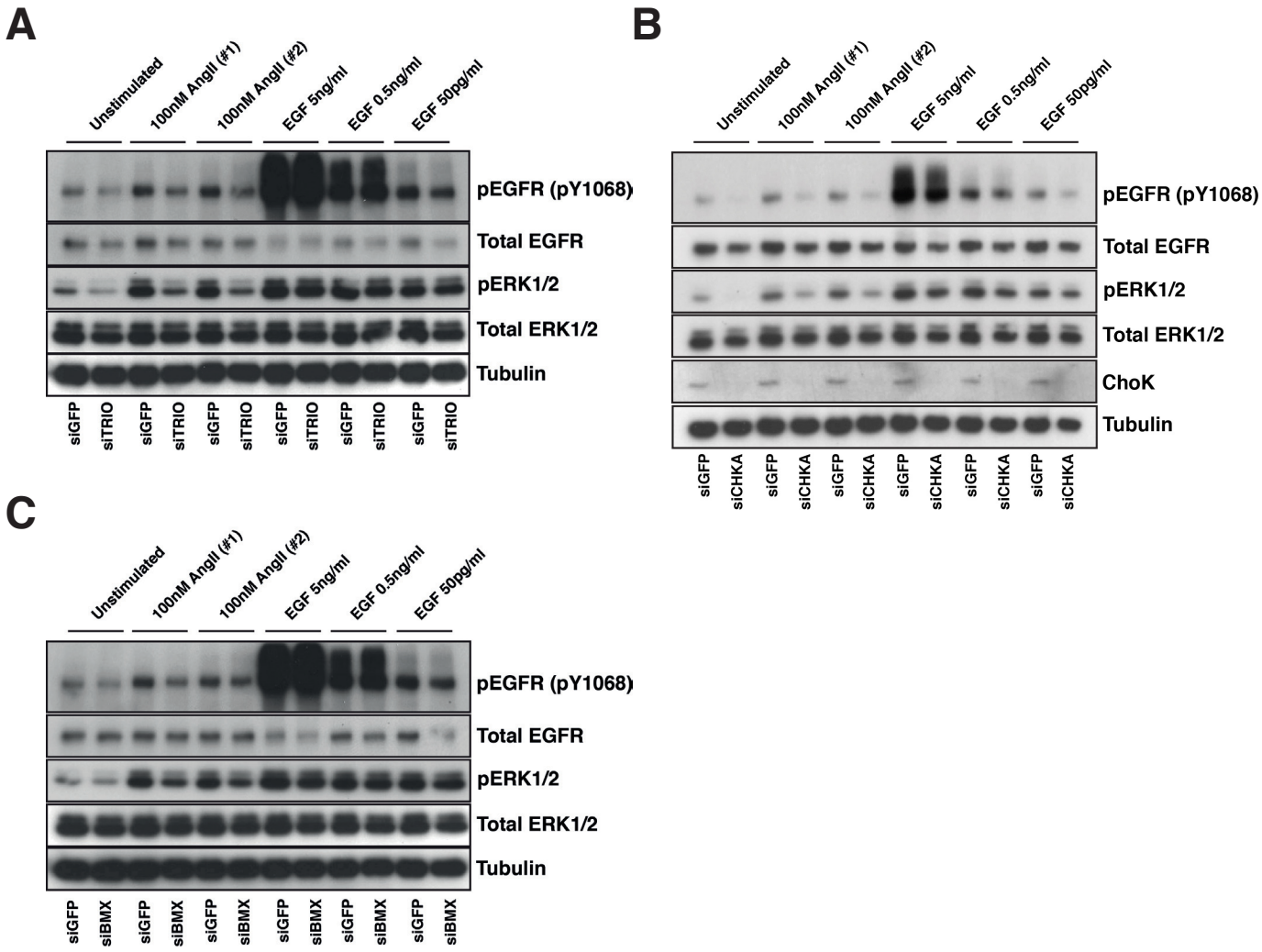

**Supplementary Figure 5: Diagram of $AT_1R$-EGFR siRNA screening workflow.** Specific details of the siRNA screening process are outlined in the MIARE which is contained within the Supplementary siRNA Screen Data file.



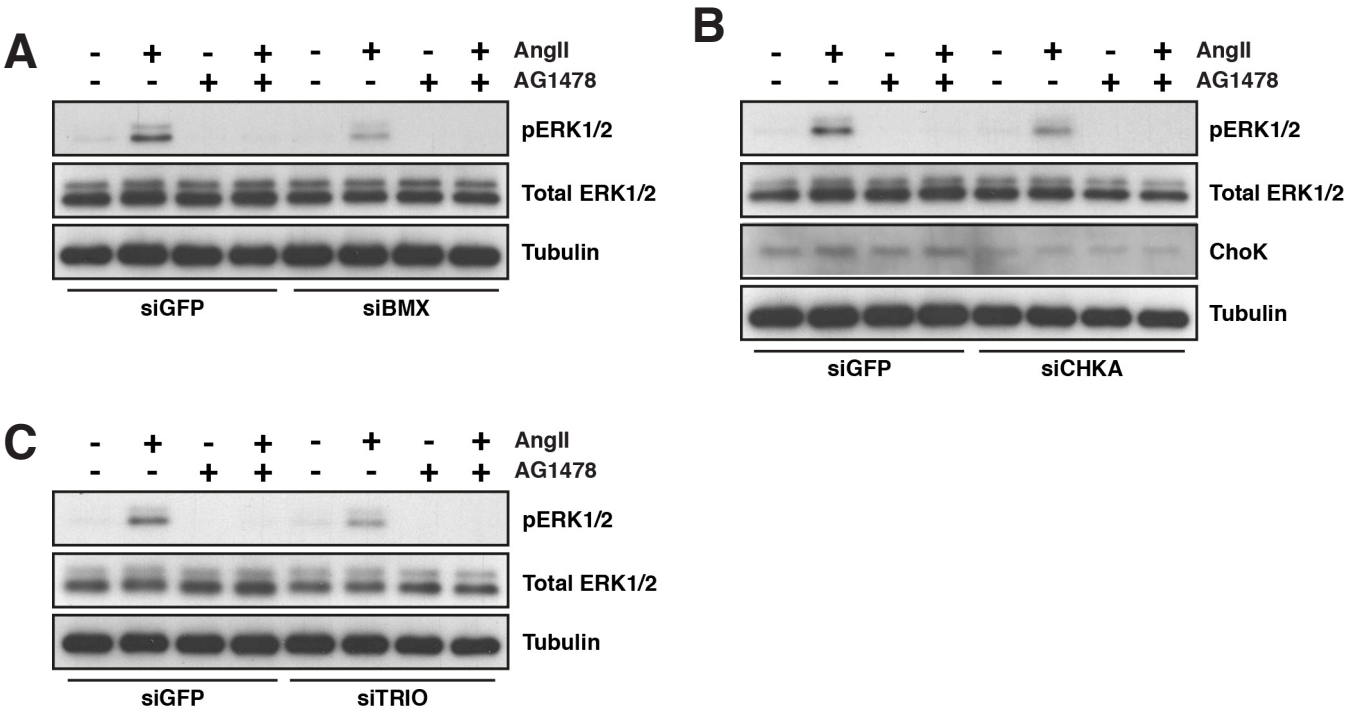

**Supplementary Figure 6: Validation of a selection of high and medium confidence hits identified from the secondary siRNA screen in the HMEC-LST-AT$_1$R cell line.** The specificity of target knockdown (using SMARTpool siRNAs) of a selection of high and medium confidence genes (CHKA, ERBB2, FN3KRP, ASK, BMX, CALM1, CDC2L2, DYRK1A, FLT3, PRKCI, CDK5, PRKCD, STYK1 and TRIO) was determined after 24 hours using qRT-PCR when compared to GFP knockdown **(A)**. Similarly, the AT$_1$R-EGFR transactivation after a 72 hour knockdown of these targets (using pEGFR (pY1068) and pERK1/2 as a readout) was also determined by western blotting **(B)** after knockdown of BMX, DYRK1A and CDC2L2 **(i)**, CALM1, ERBB2 and FLT3 **(ii)**, PRKCI, PRKCD and ASK **(iii)**, STYK1, CDK5 and TRIO **(iv)** and CHKA and FN3KRP **(v)** compared to siGFP transfected cells. The mRNA knockdown data displayed above for TRIO, CHKA and BMX is also demonstrated in Figure 5A, 5C and 5E of the manuscript, while the western blot data displayed for BMX and CHKA (B(i) and B(v) of this figure) can be viewed in their truncated form in Figure 5F and 5D of the manuscript respectively. n=3-4 experiments (for both mRNA and protein expression data); paired Student 2-tailed *t* test (compared to siGFP transfected); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, $^{ns}$ not signficant. Individual target *p* values; siCHKA ($p$=0.0002), siERBB2 ($p$=0.0026), siFN3KRP ($p$=0.0001), siASK ($p$=0.0144), siBMX ($p$=0.0008), siCALM1 ($p$=0.0005), siCDC2L2 ($p$=0.0131), siDYRK1A ($p$=0.0002), siFLT3 ($p$=0.4889), siPRKCI ($p$<0.0001), siCDK5 ($p$=0.0015), siPRKCD ($p$=0.0011), siSTYK1 ($p$=0.0222), siTRIO ($p$=0.0144).



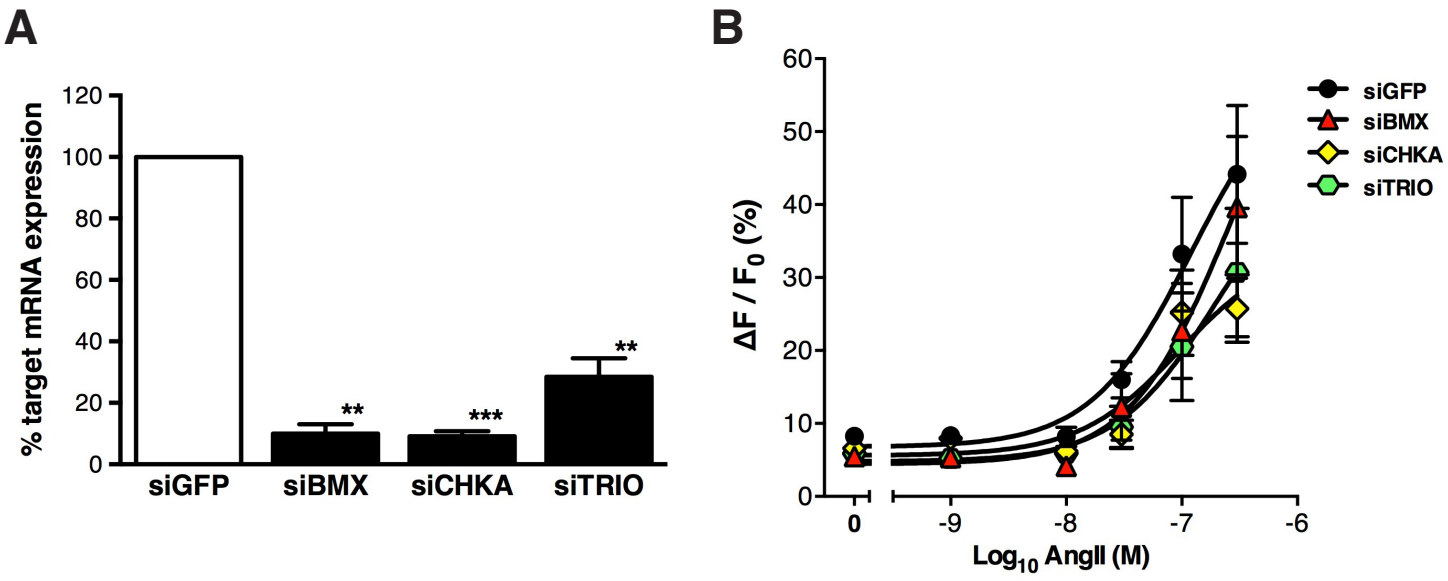

**Supplementary Figure 7: Evaluation of TRIO, BMX and CHKA individual siRNA duplex knockdown on $AT_1R$-EGFR transactivation.** To determine whether the 4 individual TRIO, BMX or CHKA siRNA duplexes (comprising the siRNA SMARTpools) were specific for each target transcript and could modulate the $AT_1R$-EGFR transactivation response, HMEC-LST-$AT_1R$ cells were reverse transfected with 25 nM of each individual siRNA duplex for either 24 hours (to assess target knockdown using qRT-PCR) or 72 hours (to assess $AT_1R$-EGFR transactivation), and treated/harvested as outlined in the Materials and Methods. We confirmed statistically significant knockdown of the TRIO transcript with all siTRIO duplexes (**A**, siTRIO-1 ($p$=0.0001), siTRIO-2 ($p$=0.0309), siTRIO-3 ($p$=0.0057), siTRIO-4 ($p$=0.0231)), and when $AT_1R$-EGFR transactivation was tested (**B**), we observed a reduction in EGFR activation with 3/4 duplexes, and ERK1/2 activation in 4/4 duplexes when compared to siGFP transfected cells. Similarly, we confirmed statistically significant knockdown of all BMX siRNA duplexes tested (**C**, siBMX-19 ($p$=0.0019), siBMX-20 ($p$=0.0066), siBMX-21 ($p$=0.0016), siBMX-22 ($p$=0.0020)), and a reduction in both EGFR and ERK1/2 activation for all (4/4) BMX duplexes tested in $AT_1R$-EGFR transactivation (compared with siGFP transfected) (**D**). We also confirmed statistically significant knockdown of 4/4 CHKA duplexes at the mRNA level - siCHKA-1 ($p$=0.0001), siCHKA-3 ($p$<0.0001), siCHKA-4 ($p$=0.0001), siCHKA-17 ($p$=0.0010) (**E**). We observed a reduction in EGFR activation with 3/4 CHKA duplexes, and ERK1/2 activation in 3/4 duplexes when compared to siGFP transfected cells, and a reduction in ChoK protein (**F**). n=3 experiments (for both mRNA and western blot data); paired Student 2-tailed *t* test (compared to siGFP transfected); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



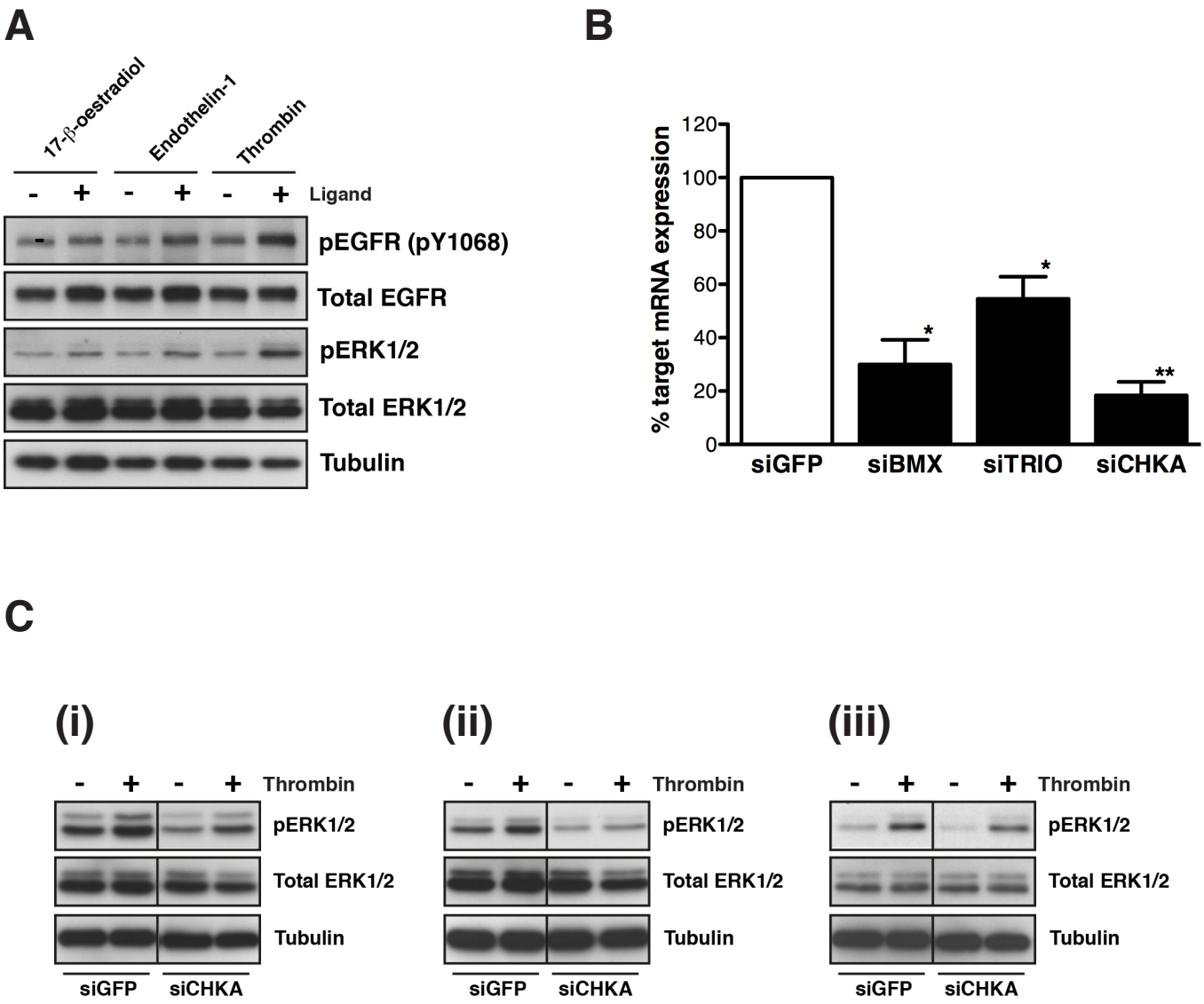

**Supplementary Figure 8: TRIO, CHKA and BMX knockdown and AngII/EGF stimulation in HMEC-LST-AT$_1$R cells.** HMEC-LST-AT$_1$R cells were reverse transfected with 40 nM of the siRNA SMARTpools (siTRIO, siCHKA, siBMX) or siGFP for 72 hours and stimulated with either 100 nM AngII (two separate batches of AngII were used to confirm our observation: AngII #1 and AngII #2), or varying doses of EGF ligand (50 pg/ml, 0.5 ng/ml and 5 ng/ml) for 10 minutes. Knockdown of TRIO (**A**), CHKA (**B**) or BMX (**C**) (compared to siGFP transfected cells) does not dramatically impact on the response of cells to EGF ligand stimulation (as determined by the activation of EGFR and ERK1/2). Data is representative of n=3 independent experiments.




**Supplementary Figure 9: TRIO, CHKA and BMX knockdown, pretreatment with AG1478 and AngII stimulation in HMEC-LST-AT$_1$R cells.** HMEC-LST-AT$_1$R cells were reverse transfected with 40 nM of the siRNA SMARTpools (siTRIO, siCHKA, siBMX) or siGFP for 72 hours, and pretreated with AG1478 (5 μM) for 30 minutes prior to stimulation with 100 nM AngII (10 minutes). Knockdown of BMX (**A**), CHKA (**B**) or TRIO (**C**) reduces AngII mediated ERK1/2 activation, and in all cases, treatment with AG1478 prevents EGFR independent ERK1/2 activation upon AngII stimulation. Data is representative of n=3 independent experiments.




**Supplementary Figure 10: Assessment of AngII-mediated calcium mobilisation with BMX, CHKA and TRIO knockdown in HMEC-LST-AT$_1$R cells.** Knockdown of BMX, TRIO and CHKA transcripts in HMEC-LST-AT$_1$R cells (using siRNA SMARTpools) was confirmed at 24 hours using qRT-PCR as described in the Materials and Methods (**A**). All siRNAs significantly knocked down their target transcript (siBMX ($p$=0.0011), siCHKA ($p$=0.0003) and siTRIO ($p$=0.0069)). For mRNA knockdown analysis, n=3 experiments; paired Student 2-tailed $t$ test (compared to siGFP transfected); ** $p < 0.01$, *** $p < 0.001$. Calcium mobilisation experiments were carried out at 72 hours knockdown of BMX, CHKA and TRIO (**B**). Data is plotted as the % change in fluorescence (ΔF) over the baseline fluorescence ($F_0$); n=2-3 independent experiments (mean ± SEM).




**Supplementary Figure 11: Assessment of GPCR-mediated EGFR transactivation in HMEC-LST cells.** HMEC-LST cells were stimulated for 10 minutes with a panel of GPCR ligands - endothelin-1 (100 nM), 17-β-oestradiol (100 nM) and thrombin (10 nM) in a pilot study to determine whether these ligands are capable of inducing activation of EGFR and ERK1/2 (**A**). Thrombin was determined to have an effect (which we demonstrate is due to transactivation of the EGFR in Figure 7F of the manuscript). Knockdown of BMX, TRIO and CHKA was performed in the HMEC-LST cells and quantified at the mRNA level at 24 hours (**B**) (corresponding thrombin-mediated EGFR transactivation data at 72 hours is located in Figure 7G of the manuscript). All siRNAs significantly knocked down their target transcript (siBMX ($p$=0.0170), siTRIO ($p$=0.0318) and siCHKA ($p$=0.0039)). To demonstrate that the effect of CHKA knockdown on thrombin-mediated ERK1/2 activation is reproducible, we present data from 3 independent experiments which demonstrates that CHKA knockdown impairs ERK1/2 activation upon thrombin stimulation (**C**) (**i**), (**ii**) and (**iii**). Data demonstrated in C(ii) of this figure is also demonstrated in Figure 7G of the manuscript. For mRNA knockdown analysis, n=3 experiments; paired Student 2-tailed *t* test (compared to siGFP transfected); * $p < 0.05$, ** $p < 0.01$.

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[1] School of Biomedical Sciences, The University of Queensland, St. Lucia, Queensland, 4072, Australia; [2] Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, Victoria, 3010, Australia; [3] Oncogenic Signalling and Growth Control Program, Peter MacCallum Cancer Centre, East Melbourne, Victoria, 3002, Australia; [4] The Victorian Centre for Functional Genomics, Peter MacCallum Cancer, East Melbourne, Victoria, 3002, Australia; [5] Department of Pathology, The University of Melbourne, Parkville, Victoria, 3010, Australia; [6] Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, 3004, Australia; [7] Department of Biochemistry and Molecular Biology, The University of Melbourne, Parkville, Victoria, 3010, Australia; [8] Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, 3800, Australia.

## Supplementary Table S1

**Download Table S1**

# A functional siRNA screen identifies genes modulating angiotensin II-mediated EGFR transactivation

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## Supplementary Table S2

**Download Table S2**

# A functional siRNA screen identifies genes modulating angiotensin II-mediated EGFR transactivation

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## Supplementary Table S3

## Supplementary Table S3: Secondary screen data

| Entrez Gene ID | Entrez Gene Name | Duplex # | Average (Mock+GFP) Fold Change | Duplex hits (/4) |
|---|---|---|---|---|
| 207 | AKT1 | D-003000-05 | -2.054 | |
| 207 | AKT1 | D-003000-07 | -1.542 | |
| 207 | AKT1 | D-003000-08 | -2.333 | |
| 207 | AKT1 | D-003000-22 | -1.407 | 3 |
| 10000 | AKT3 | D-003002-10 | -0.196 | |
| 10000 | AKT3 | D-003002-09 | 1.397 | |
| 10000 | AKT3 | D-003002-11 | -2.331 | |
| 10000 | AKT3 | D-003002-12 | -1.831 | 2 |
| 10926 | ASK | D-004165-01 | 1.640 | |
| 10926 | ASK | D-004165-03 | -2.002 | |
| 10926 | ASK | D-004165-06 | -1.876 | |
| 10926 | ASK | D-004165-22 | -1.856 | 3 |
| 657 | BMPR1A | D-004933-03 | -1.369 | |
| 657 | BMPR1A | D-004933-07 | -1.228 | |
| 657 | BMPR1A | D-004933-08 | -1.494 | |
| 657 | BMPR1A | D-004933-24 | -1.762 | 1 |
| 660 | BMX | D-003106-19 | -2.274 | |
| 660 | BMX | D-003106-20 | 1.883 | |
| 660 | BMX | D-003106-21 | -1.891 | |
| 660 | BMX | D-003106-22 | -2.470 | 3 |
| 64768 | C9ORF12 | D-006703-01 | 1.969 | |
| 64768 | C9ORF12 | D-006703-03 | -1.355 | |
| 64768 | C9ORF12 | D-006703-05 | -1.510 | |
| 64768 | C9ORF12 | D-006703-06 | -2.391 | 2 |
| 801 | CALM1 | D-017646-01 | -2.343 | |
| 801 | CALM1 | D-017646-02 | -0.002 | |
| 801 | CALM1 | D-017646-03 | -2.463 | |
| 801 | CALM1 | D-017646-04 | -1.801 | 3 |
| 728642 | CDC2L2 | D-181567-09 | -2.910 | |
| 728642 | CDC2L2 | D-181567-10 | 1.536 | |
| 728642 | CDC2L2 | D-181567-11 | -3.140 | |
| 728642 | CDC2L2 | D-181567-12 | -1.530 | 3 |

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| Entrez Gene ID | Entrez Gene Name | Duplex # | Average (Mock+GFP) Fold Change | Duplex hits (/4) |
|---|---|---|---|---|
| 1020 | CDK5 | D-003239-05 | -1.339 | |
| 1020 | CDK5 | D-003239-06 | 1.418 | |
| 1020 | CDK5 | D-003239-07 | -1.822 | |
| 1020 | CDK5 | D-003239-08 | -1.796 | 2 |
| 8851 | CDK5R1 | D-008988-01 | 1.769 | |
| 8851 | CDK5R1 | D-008988-02 | 1.391 | |
| 8851 | CDK5R1 | D-008988-03 | -1.936 | |
| 8851 | CDK5R1 | D-008988-17 | -1.351 | 1 |
| 1119 | CHKA | D-006704-01 | -1.864 | |
| 1119 | CHKA | D-006704-03 | -2.020 | |
| 1119 | CHKA | D-006704-17 | -3.025 | |
| 1119 | CHKA | D-006704-04 | -1.777 | 4 |
| 80347 | COASY | D-006751-01 | -1.418 | |
| 80347 | COASY | D-006751-02 | 0.228 | |
| 80347 | COASY | D-006751-03 | 1.384 | |
| 80347 | COASY | D-006751-04 | 1.768 | 1 |
| 1399 | CRKL | D-012023-01 | -1.928 | |
| 1399 | CRKL | D-012023-02 | 0.102 | |
| 1399 | CRKL | D-012023-18 | 0.038 | |
| 1399 | CRKL | D-012023-05 | 0.055 | 1 |
| 53944 | CSNK1G1 | D-004666-05 | -1.181 | |
| 53944 | CSNK1G1 | D-004666-06 | 1.759 | |
| 53944 | CSNK1G1 | D-004666-07 | -1.480 | |
| 53944 | CSNK1G1 | D-004666-08 | -1.131 | 1 |
| 1460 | CSNK2B | D-007679-01 | 1.241 | |
| 1460 | CSNK2B | D-007679-02 | -3.172 | |
| 1460 | CSNK2B | D-007679-03 | -1.427 | |
| 1460 | CSNK2B | D-007679-04 | -1.617 | 2 |
| 160851 | DGKH | D-006716-01 | -1.588 | |
| 160851 | DGKH | D-006716-02 | -0.085 | |
| 160851 | DGKH | D-006716-03 | -1.311 | |
| 160851 | DGKH | D-006716-04 | -2.683 | 2 |
| 1739 | DLG1 | D-009415-01 | -2.198 | |
| 1739 | DLG1 | D-009415-02 | -1.272 | |
| 1739 | DLG1 | D-009415-03 | 1.233 | |
| 1739 | DLG1 | D-009415-04 | 1.284 | 1 |
| 1859 | DYRK1A | D-004805-02 | -1.642 | |

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| Entrez Gene ID | Entrez Gene Name | Duplex # | Average (Mock+GFP) Fold Change | Duplex hits (/4) |
|---|---|---|---|---|
| 1859 | DYRK1A | D-004805-01 | 1.550 | |
| 1859 | DYRK1A | D-004805-04 | -2.200 | |
| 1859 | DYRK1A | D-004805-17 | -1.996 | 3 |
| 1956 | EGFR | D-003114-06 | -3.711 | |
| 1956 | EGFR | D-003114-22 | -1.556 | |
| 1956 | EGFR | D-003114-08 | -1.464 | |
| 1956 | EGFR | D-003114-23 | -1.557 | 3 |
| 2049 | EPHB3 | D-003123-09 | 0.026 | |
| 2049 | EPHB3 | D-003123-10 | -1.249 | |
| 2049 | EPHB3 | D-003123-11 | -2.536 | |
| 2049 | EPHB3 | D-003123-12 | 1.220 | 1 |
| 2064 | ERBB2 | D-003126-07 | -1.501 | |
| 2064 | ERBB2 | D-003126-06 | -1.862 | |
| 2064 | ERBB2 | D-003126-05 | -2.781 | |
| 2064 | ERBB2 | D-003126-08 | -1.590 | 4 |
| 2242 | FES | D-003130-05 | -2.625 | |
| 2242 | FES | D-003130-06 | 1.204 | |
| 2242 | FES | D-003130-07 | -2.227 | |
| 2242 | FES | D-003130-08 | -2.513 | 3 |
| 2261 | FGFR3 | D-003133-05 | -0.106 | |
| 2261 | FGFR3 | D-003133-06 | -1.570 | |
| 2261 | FGFR3 | D-003133-07 | -3.692 | |
| 2261 | FGFR3 | D-003133-08 | 1.277 | 2 |
| 2322 | FLT3 | D-003137-06 | -1.780 | |
| 2322 | FLT3 | D-003137-05 | -3.171 | |
| 2322 | FLT3 | D-003137-07 | -1.501 | |
| 2322 | FLT3 | D-003137-08 | -1.219 | 3 |
| 79672 | FN3KRP | D-006817-02 | -1.570 | |
| 79672 | FN3KRP | D-006817-01 | -2.932 | |
| 79672 | FN3KRP | D-006817-03 | -2.421 | |
| 79672 | FN3KRP | D-006817-04 | -3.943 | 4 |
| 2444 | FRK | D-003139-05 | -0.007 | |
| 2444 | FRK | D-003139-06 | 1.102 | |
| 2444 | FRK | D-003139-07 | -2.574 | |
| 2444 | FRK | D-003139-08 | -1.555 | 2 |
| 6011 | GRK1 | D-004662-01 | 1.352 | |
| 6011 | GRK1 | D-004662-03 | 1.279 | |

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| Entrez Gene ID | Entrez Gene Name | Duplex # | Average (Mock+GFP) Fold Change | Duplex hits (/4) |
|---|---|---|---|---|
| 6011 | GRK1 | D-004662-02 | 1.406 | |
| 6011 | GRK1 | D-004662-04 | -1.174 | 0 |
| 10114 | HIPK3 | D-004810-01 | -1.174 | |
| 10114 | HIPK3 | D-004810-02 | 1.055 | |
| 10114 | HIPK3 | D-004810-03 | 1.258 | |
| 10114 | HIPK3 | D-004810-04 | 2.552 | 1 |
| 51347 | JIK | D-004844-01 | 1.304 | |
| 51347 | JIK | D-004844-02 | -1.114 | |
| 51347 | JIK | D-004844-04 | 0.042 | |
| 51347 | JIK | D-004844-05 | -1.516 | 1 |
| 92335 | LYK5 | D-005343-02 | -1.141 | |
| 92335 | LYK5 | D-005343-03 | 1.455 | |
| 92335 | LYK5 | D-005343-05 | -2.064 | |
| 92335 | LYK5 | D-005343-06 | -1.272 | 1 |
| 1326 | MAP3K8 | D-003511-07 | -1.354 | |
| 1326 | MAP3K8 | D-003511-08 | -1.481 | |
| 1326 | MAP3K8 | D-003511-09 | 1.681 | |
| 1326 | MAP3K8 | D-003511-10 | -1.569 | 1 |
| 5594 | MAPK1 | D-003555-01 | -1.077 | |
| 5594 | MAPK1 | D-003555-03 | -1.771 | |
| 5594 | MAPK1 | D-003555-04 | 1.278 | |
| 5594 | MAPK1 | D-003555-09 | -1.610 | 2 |
| 9833 | MELK | D-004029-01 | -1.286 | |
| 9833 | MELK | D-004029-02 | 1.129 | |
| 9833 | MELK | D-004029-04 | -1.362 | |
| 9833 | MELK | D-004029-05 | -1.620 | 1 |
| 55750 | MULK | D-007256-01 | 1.160 | |
| 55750 | MULK | D-007256-02 | 1.291 | |
| 55750 | MULK | D-007256-04 | 1.220 | |
| 55750 | MULK | D-007256-03 | -1.759 | 1 |
| 5290 | PIK3CA | D-003018-07 | -1.518 | |
| 5290 | PIK3CA | D-003018-08 | -1.773 | |
| 5290 | PIK3CA | D-003018-25 | 1.603 | |
| 5290 | PIK3CA | D-003018-24 | 2.273 | 2 |
| 5297 | PIK4CA | D-006776-02 | 1.758 | |
| 5297 | PIK4CA | D-006776-25 | 1.271 | |
| 5297 | PIK4CA | D-006776-09 | -1.823 | |

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| Entrez Gene ID | Entrez Gene Name | Duplex # | Average (Mock+GFP) Fold Change | Duplex hits (/4) |
|---|---|---|---|---|
| 5297 | PIK4CA | D-006776-03 | -2.198 | 2 |
| 8395 | PIP5K1B | D-004058-01 | 2.424 | |
| 8395 | PIP5K1B | D-004058-02 | -1.577 | |
| 8395 | PIP5K1B | D-004058-04 | 1.161 | |
| 8395 | PIP5K1B | D-004058-03 | 1.123 | 1 |
| 200576 | PIP5K3 | D-005058-09 | -0.019 | |
| 200576 | PIP5K3 | D-005058-10 | 1.416 | |
| 200576 | PIP5K3 | D-005058-11 | -1.556 | |
| 200576 | PIP5K3 | D-005058-12 | -1.627 | 2 |
| 1263 | PLK3 | D-003257-05 | -1.125 | |
| 1263 | PLK3 | D-003257-07 | -1.039 | |
| 1263 | PLK3 | D-003257-08 | 1.217 | |
| 1263 | PLK3 | D-003257-09 | -2.127 | 1 |
| 5562 | PRKAA1 | D-005027-01 | 1.323 | |
| 5562 | PRKAA1 | D-005027-02 | -1.061 | |
| 5562 | PRKAA1 | D-005027-03 | -2.002 | |
| 5562 | PRKAA1 | D-005027-05 | -2.166 | 2 |
| 5580 | PRKCD | D-003524-03 | 1.083 | |
| 5580 | PRKCD | D-003524-04 | 0.030 | |
| 5580 | PRKCD | D-003524-05 | -1.999 | |
| 5580 | PRKCD | D-003524-06 | -2.491 | 2 |
| 5583 | PRKCH | D-004655-02 | -0.013 | |
| 5583 | PRKCH | D-004655-03 | 1.673 | |
| 5583 | PRKCH | D-004655-04 | -1.238 | |
| 5583 | PRKCH | D-004655-05 | -2.419 | 1 |
| 5584 | PRKCI | D-004656-02 | 1.475 | |
| 5584 | PRKCI | D-004656-03 | -2.055 | |
| 5584 | PRKCI | D-004656-06 | -1.609 | |
| 5584 | PRKCI | D-004656-05 | -1.911 | 3 |
| 5592 | PRKG1 | D-004658-01 | -1.315 | |
| 5592 | PRKG1 | D-004658-03 | -1.210 | |
| 5592 | PRKG1 | D-004658-04 | -1.738 | |
| 5592 | PRKG1 | D-004658-09 | 1.338 | 1 |
| 85481 | PSKH2 | D-005366-01 | -1.727 | |
| 85481 | PSKH2 | D-005366-02 | 1.576 | |
| 85481 | PSKH2 | D-005366-03 | -2.118 | |
| 85481 | PSKH2 | D-005366-04 | -1.041 | 2 |

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| Entrez Gene ID | Entrez Gene Name | Duplex # | Average (Mock+GFP) Fold Change | Duplex hits (/4) |
|---|---|---|---|---|
| 8986 | RPS6KA4 | D-004664-01 | -1.091 | |
| 8986 | RPS6KA4 | D-004664-02 | 1.213 | |
| 8986 | RPS6KA4 | D-004664-03 | -2.660 | |
| 8986 | RPS6KA4 | D-004664-04 | 1.254 | 1 |
| 54861 | SNRK | D-004322-01 | -1.910 | |
| 54861 | SNRK | D-004322-17 | -2.168 | |
| 54861 | SNRK | D-004322-04 | -0.039 | |
| 54861 | SNRK | D-004322-18 | 1.278 | 2 |
| 8576 | STK16 | D-004054-01 | -1.406 | |
| 8576 | STK16 | D-004054-03 | -1.436 | |
| 8576 | STK16 | D-004054-02 | -1.213 | |
| 8576 | STK16 | D-004054-04 | -1.787 | 1 |
| 55359 | STYK1 | D-003113-05 | 1.134 | |
| 55359 | STYK1 | D-003113-08 | -2.069 | |
| 55359 | STYK1 | D-003113-07 | -2.955 | |
| 55359 | STYK1 | D-003113-06 | -1.224 | 2 |
| 7204 | TRIO | D-005047-01 | 1.232 | |
| 7204 | TRIO | D-005047-03 | 0.009 | |
| 7204 | TRIO | D-005047-02 | -1.649 | |
| 7204 | TRIO | D-005047-04 | -2.217 | 2 |

# A functional siRNA screen identifies genes modulating angiotensin II-mediated EGFR transactivation

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## Supplementary Table S4

## Supplementary Table S4: Primer sequences used for qRT-PCR analysis

| mRNA | Forward (5'-3') | Reverse (5'-3') | Amplicon (bp) | RefSeq | Exon-Exon |
|---|---|---|---|---|---|
| Agtr1a (rat)* | AGATCGCTTCGGCCAGCGTG | TTGGCCACCAGCATCGTGCG | 133 | NM_030985.4 | N |
| ASK (DBF4) | AGACGCGGTACCTCTACTGCGT | TGGGTCCGGAGAAAGTGTCGCA | 124 | NM_006716.3 | N |
| BMX | CTCCGCCACCCTGTGTCAACAA | TTGCCCAGCTGGACCACTCCAA | 143 | NM_001721.6 | Y |
| CALM1 | CCAAATGGGTGCATTCAGGGCT | AAGCACATGGGCGATGTCTCCC | 147 | NM_006888.4 | N |
| CDC2L2 | ACGGGCTTCCACCTTACCACCA | TCCGAGTCTCATCGCAGCTCCA | 157 | NM_024011.2 | N |
| CDK5 | GCCGCAATGTGCTACACAGGGA | CACAGTGTGACCACCTCAGCTGA | 142 | NM_004935.3 | Y |
| CHKA | ACCTGACACCACAGCCACCCTT | ACCATGGCCTCAGCCCCTTGAA | 147 | NM_001277.2 | Y |
| DYRK1A | TGACCGTCGCCAGCCAAACA | TGGGGCATCCGCCTCTGTAACA | 126 | NM_001396.3 | Y |
| EGFR | GGCTGTCCAACGAATGGGCCTAAG | GCGTGCGCTTCCGAACGATG | 133 | NM_005228.3 | Y |
| ERBB2 | AAGTGTGCACCGGCACAGACA | TGGCATTGGTGGGCAGGTAGGT | 138 | NM_004448.2 | Y |
| FLT3 | TGGACAGTGGGTGTCGAGCAGT | AAGGGAAGGGGCCTGGAGAGTT | 134 | NM_004119.2 | Y |
| FN3KRP | AGCTTGGAGAGATGCGCCTGAA | TCCTGCCAGTCATTCACCTGGG | 139 | NM_024619.3 | Y |
| GAPD | GGACTCATGACCACAGTCCATGCC | ATGACCTTGCCCACAGCCTTGG | 146 | NM_002046.3 | Y |
| GNAQ | GATGTTCGTGGACCTGAACC | ACTGGAGGATGGTGTCCTTG | 119 | NM_002072.3 | N |
| PRKCD | TGGTGGTTGGTGCGTTGTAGCA | TTGAAGGCGATGCGCAGGAACG | 116 | NM_006254.3 | Y |
| PRKCI | ACGTCCTGGGATGCCTTGTCCA | TGAGCACGCCTGTTGAAACGCT | 126 | NM_002740.5 | Y |
| STYK1 | CTGTCGGCGGGATGTGATGACT | GCTGCCACATCCCCATGGAACA | 138 | NM_018423.2 | Y |
| RAC1 | TCCGCAAACAGATGTGTTCTTAAT | ATGGGAGTGTTGGGACAGTG | 114 | NM_006908.4 | Y |
| RHOA | CGTTAGTCCACGGTCTGGTC | GCCATTGCTCAGGCAACGAA | 115 | NM_001664.2 | Y |
| TRIO | GGCCTTCTTCCGATCCGGGTT | GATTGCTGCGGGCCGGAAAC | 146 | NM_007118.2 | Y |

**Note:** Primers were designed over exon-exon junctions (Y) where possible; primers not spanning exon-exon junctions (N).

* Detects HA-tagged $AT_1R$ (rat origin)