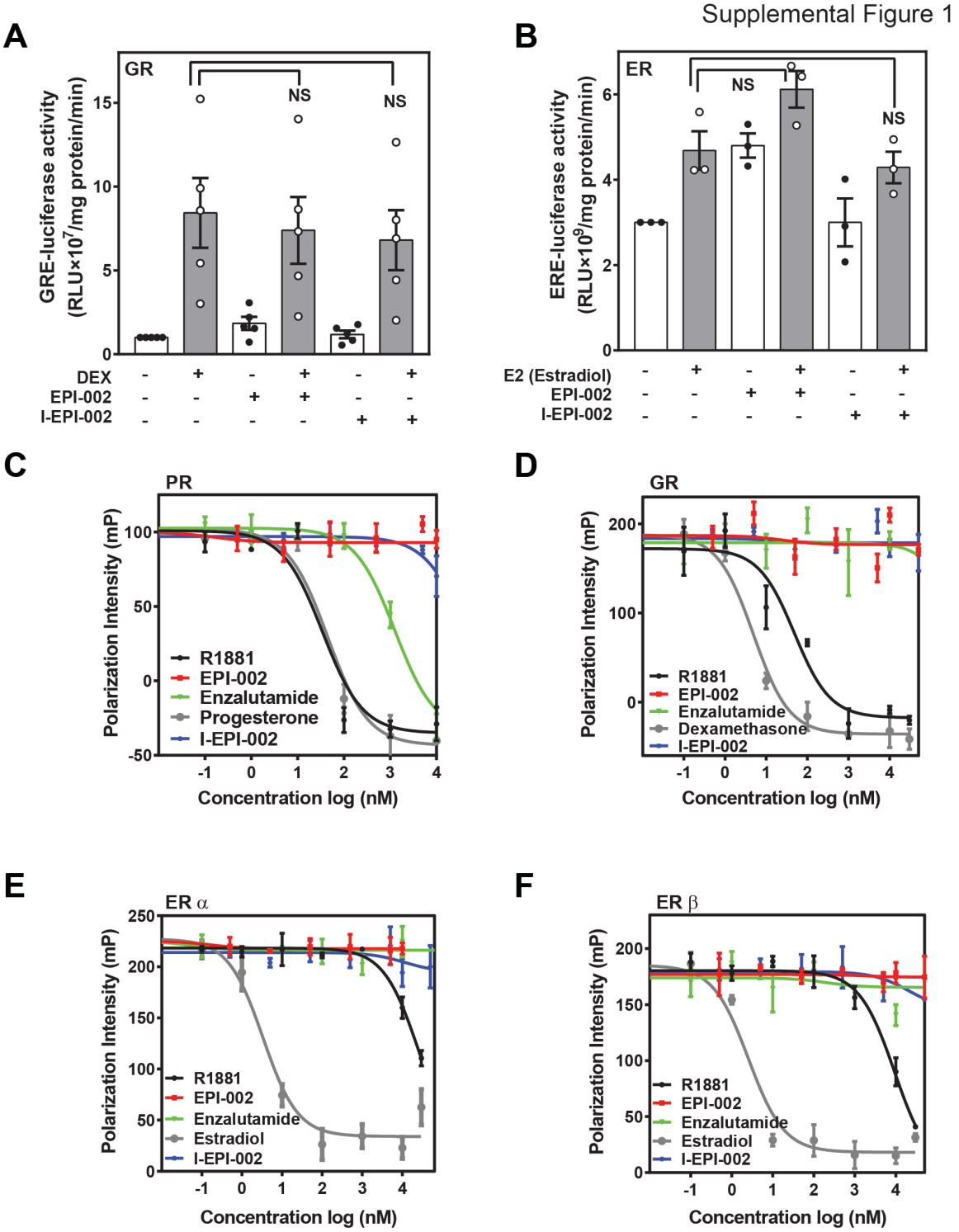
Supplemental Figure 1



Supplemental Figure Legends

Supplemental Figure 1.

(**A**) Effect of I-EPI-002, EPI-002 or enzalutamide on dexamethasone (DEX, 10 nM) induced GR transcriptional activity in LNCaP cells that were transiently transfected with GRE-luciferase reporter and expression vector for GR. (**B**) Effect of I-EPI-002, EPI-002 or enzalutamide on estradiol (E2, 10 nM) induced ER transcriptional activity in LNCaP cells that were transiently transfected with ERE-luciferase reporter and expression vector for ER. (**C**) I-EPI-002 and EPI-002 did not inhibit PR ligand-binding, whereas enzalutamide did. (**D**) I-EPI-002, EPI-002 and enzalutamide did not inhibit GR ligand-binding. (**E**) I-EPI-002, EPI-002 and enzalutamide did not inhibit ERα ligand-binding. (**F**) I-EPI-002, EPI-002 and enzalutamide did not inhibit ERβ ligand-binding. For (**A**) and (**B**), values are shown as mean ± SEM from n = 5 for GR and n = 3 for ER with each experiment performed in triplicate. NS: not statistically significant. One-way ANOVA Dunnett's multiple comparison test was performed. For (**C**) to (**F**), representative competitive binding curves are shown from experiments that were repeated 3 times.

Supplemental Experimental Procedures

I) Synthesis of Cold 15-IodoEPI002 (6) Reference Material

Preparation of (*R*)-2,2-Dimethyl-1,3-dioxolane-4-methanol *p*-toluenesulfonate 1: *p*-Toluenesulfonyl chloride (6.5 g, 34.1 mmol) was added portion-wise over a period of 10 min to a solution of (*S*)-(+)-1,2-isopropylideneglycerol (3.0 g, 22.7 mmol) and DMAP (30mg, 0.25mmol) in anhydrous pyridine (30 mL). The resulting solution was stirred overnight at rt. The pyridine was removed under reduced pressure and the residue was diluted with ethyl acetate (50 mL) and washed sequentially with water (2 x 40mL), cold aqueous 1M HCl (40 mL), saturated $NaHCO_3$ (40 mL), and finally water (40 mL). The organic layer was dried over Mg_2SO_4, filtered, and concentrated to give a light yellow oil. The crude oil was purified by Si gel gradient flash column chromatography (eluent: 10% ethyl acetate in hexane to 30% ethyl acetate in hexane) to afford (*R*)-2,2-dimethyl-1,3-dioxolane-4-methanol *p*-toluenesulfonate **1** (5.91 g, 91 % yield) as a colorless viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.77-7.79 (2H, d, J = 8.4), 7.26-7.35 (2H, d, J = 8.0), 4.23-4.29 (1H, m), 3.93-4.04 (3H, m), 3.72-3.76 (1H, dd, J =8.8, 5.2), 2.43 (3H, s), 1.32 (3H, s), 1.29 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 145.22, 132.75, 130.06, 128.11, 110.15, 73.04, 69.66, 66.26, 26.75, 25.27, 21.77; LRMS (ESI) *m/z* $[M+Na]^+$ 309.2 [calc'd for $C_{13}H_{18}O_5SNa$ 309.1].

Preparation of Acetonide 3: Sodium hydride (60% dispersion in mineral oil, 2.27g, 56.7 mmol, 1.0 equiv.) was added slowly to a stirred solution of bisphenol A **2** (12.94 g, 56.7 mmol) in anhydrous *N,N*-dimethylformamide (60 mL) at rt and the contents were stirred

under an atmosphere of argon for 20 min. (*R*)-2,2-Dimethyl-1,3-dioxolane-4-methanol p-toluenesulfonate **1** (8.53g, 28.3 mmol, 0.5 equiv.) was added and the mixture was allowed to react at 50-60 °C for 16 h. Caution! Using sodium hydride in warmed *N, N*-dimethylformamide could result in deflagration and fire. The reaction was quenched by the addition of a saturated solution of ammonium chloride (10 mL) and the mixture was extracted with ethyl acetate (3 x 50 mL). The organic layer was washed with deionized water (3 x 40 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was purified by gradient flash Si gel column chromatography (eluent: 5% ethyl acetate in hexane to 10% ethyl acetate in hexane) to provide acetonide **3** (8.10g, 84 %) as a sticky oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.09-7.13 (4H, m), 6.82-6.85 (4H, m), 5.87 (1H, s), 4.45-4.50 (1H, m), 4.15-4.20 (1H, dd, ^{2}J = 8.8, 6.8), 4.04-4.08 (1H, dd, J=9.6, 5.6), 3.89-3.95 (2H, m), 1.61 (6H, s), 1.47 (3H, s), 1.41 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 156.86, 145.78, 144.77, 142.08, 127.79, 126.86, 120.69, 114.30, 109.90, 74.15, 68.93, 67.05, 42.08, 30.92, 26.94, 25.50; LRMS (ESI) *m/z* $[M+Na]^+$ 365.4 [calc'd for $C_{21}H_{26}O_4Na$ 365.2].

Preparation of Iodoacetonide 4: Acetonide **3** (200 mg, 0.58 mmol) was dissolved in 4 mL of methanol. One equivalent of sodium iodide (85 mg, 0.58 mmol) and 1.5 equiv. of sodium hydroxide (35 mg, 0.88 mmol) were added and the solution was cooled to 0 ˚C. Aqueous sodium hypochlorite (800 mg, 0.58 mmol of sodium hypochlorite, 1 equiv.) was then added dropwise over 2 min at 0–3 ˚C. The pH was kept between 6 and 7 by adding 10% HCl. The mixture was extracted with dichloromethane (2 x 20 mL). The organic layer was washed with deionized water (2 x 20 mL), dried over anhydrous magnesium

sulfate, filtered, and concentrated under reduced pressure to provide iodoacetonide **4** as a sticky oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.51-7.52 (1H, d, J=2.4), 7.10-7.13 (2H, m), 7.03-7.06 (1H, dd, J=8.4, 2.0), 6.85-6.87 (1H, d, J=8.4), 6.81-6.83 (2H, m), 5.37 (1H, s), 4.46-4.49 (1H, m), 4.10-4.19 (1H, m), 4.03-4.07 (1H, m), 3.89-3.94 (2H, m), 1.61 (6H, s), 1.47 (3H, s), 1.41 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 156.67, 152.86, 145.42, 142.97, 136.25, 129.11, 127.83, 114.63, 114.15, 109.88, 85.52, 74.16, 68.91, 67.06, 41.74, 31.11, 26.94, 25.51; LRMS (ESI) *m/z* $[M+Na]^+$ 491.2 [calc'd for $C_{21}H_{25}IO_4Na$ 491.1].

Preparation of Iodoepoxide 5: Sodium hydride (60% dispersion in mineral oil, 41.6 mg, 1.04 mmol, 2.0 equiv.) was added slowly to a stirred solution of iodoacetonide **4** in anhydrous *N,N*-dimethyl formamide (3 mL) at rt and the contents were stirred under an atmosphere of argon for 10 min. A solution of (2*R*)-(-)-glycidyl tosylate 98% (142 mg, 0.62 mmol, 1.5 equiv.) in anhydrous *N,N*-dimethyl formamide (2 mL) was added via syringe and the mixture was allowed to react at 65-70 ˚C for 40 min. The reaction was quenched by the addition of a saturated solution of ammonium chloride (1 mL), and the mixture was extracted with dichloromethane (2 x 20 mL). The organic layer was washed with deionized water (2 x 20 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to provide iodoepoxide **5** that was used in the next step without purification**.**

Preparation of 15-IodoEPI-002 6: To a solution of iodoepoxide **5** in acetonitrile (15 mL) was added $CeCl_3 \cdot 7H_2O$ (391 mg, 1.05 mmol, 2.5 equiv.) and the mixture was refluxed for 1 h. The resulting white paste was collected by filtration and washed with

dichloromethane and the clear suspension was concentrated under reduced pressure. The resulting residue was purified by gradient flash column chromatography on Si gel (eluent: 25% ethyl acetate in hexane to 70% ethyl acetate in hexane) to provide 15-iodoEPI002 **6** (59 mg, 20 % for 3 steps from acetonide **3**) as a sticky oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.62-7.63 (1H, d, J=2.0), 7.10-7.13 (3H, m), 6.81-6.84 (2H, m), 6.71-6.73 (1H, d, J=8.4), 4.21-4.24 (1H, m), 4.06-4.17 (3H, m), 4.02-4.04 (2H, m), 3.72-3.86 (4H, m), 1.62 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 156.54, 154.60, 146.40, 143.09, 137.67, 128.30, 127.91, 114.20, 112.05, 86.67, 70.51, 69.92, 69.74, 69.30, 63.82, 45.85, 41.82, 31.07; LRMS (ESI) *m/z* $[M+Na]^+$ 543.1 [calc'd for $C_{21}H_{26}C_lIO_5Na$ 543.0].

II) Radiosynthesis of [^{123}I]15-IodoEPI002

Preparation of [^{123}I]Iodoacetonide 7: 50 µL phosphate buffer (1 M, pH 6.0), 0.2 mg acetonide **3** in 100 µL methanol, and 0.15 mg chloramine-T in 100 µL water were added to the [^{123}I]NaI solution from Nordion. The mixture was incubated at rt for 5 min, diluted with 100 µL methanol, and purified by HPLC using the Phenomenex Luna semipreparative column eluting with 66% acetonitriel/34% water at a flow rate of 4.5 mL/min. The retention time of [^{123}I] iodoacetonide **7** was 17.8 min. The eluting fraction containing [^{123}I] iodoacetonide **7** was collected, diluted with water (50 mL), and passed through a Waters C18 light Sep-Pak cartridge. The trapped [^{123}I isodoacetonide **7** on the Sep-Pak cartridge was washed out with tetrahydrofuran (0.5 mL) and the solvent was removed with a helium stream to give a residue of **7** that was used in the next step.

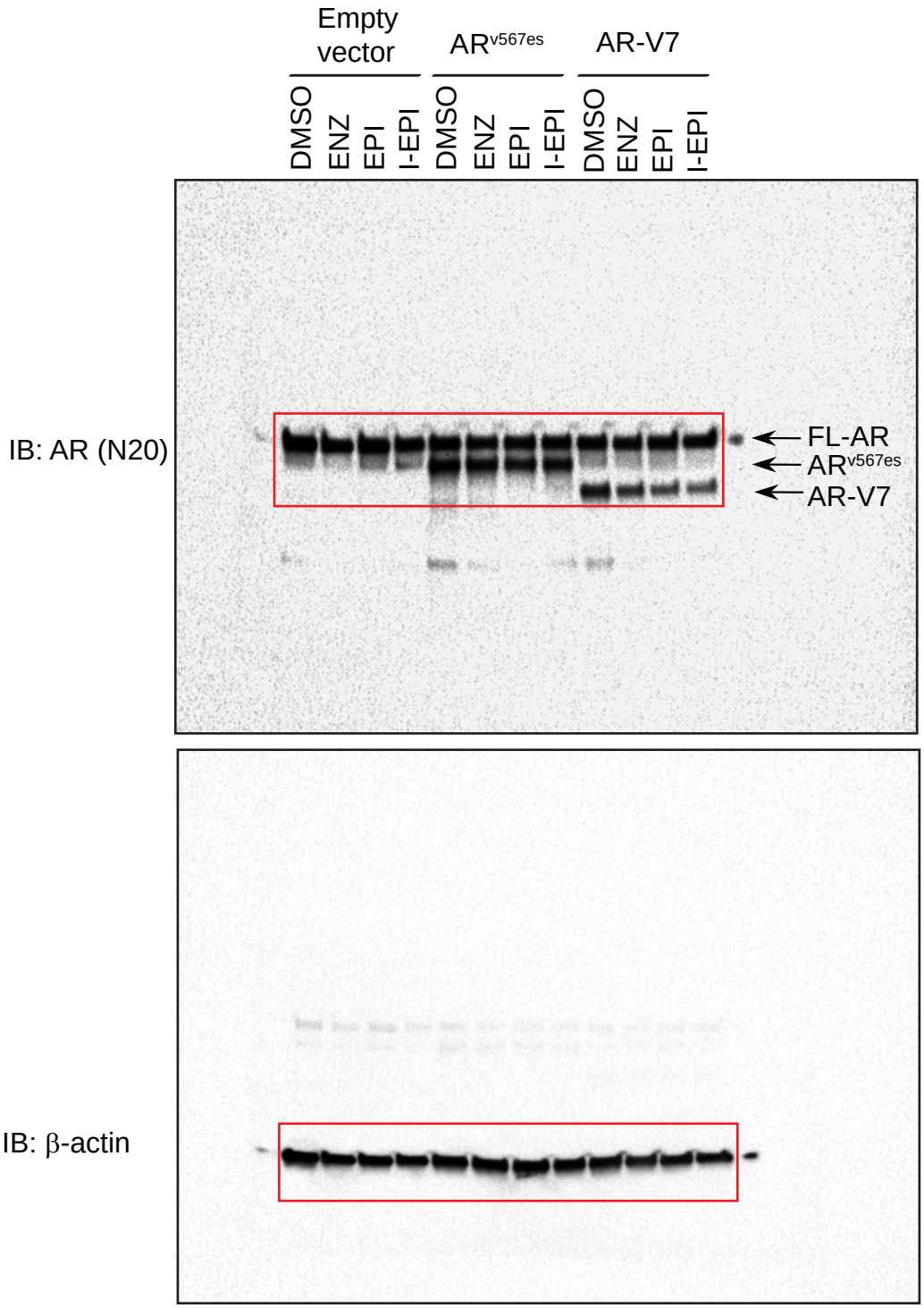
Preparation of [^{123}I] Iodoepoxide 8: To the dried residue of [^{123}I] iodoacetonide **7** was

added to 10 mg of sodium hydride (60% dispersion in mineral oil) and 20 mg (2R)-(-)-glycidyl tosylate dissolved in 500 μL anhydrous *N,N*-dimethyl formamide. The mixture was heated at 70 °C for 60 min. At the end of heating, the mixture was diluted with saturated ammonium chloride (250 μL) and water (250 μL) and purified by HPLC using the Phenomenex Luna semipreparative column eluting with 75% acetonitrile/25% water at a flow rate of 4.5 mL/min. The retention time of the desired [^{123}I]iodoepoxide **8** was 15.8 min. The eluting fraction containing [^{123}I]iodoepoxide **8** was collected, diluted with water (50 mL), and passed through a Waters C18 light Sep-Pak cartridge. The trapped [^{123}I]iodoepoxide **8** on the Sep-Pak cartridge was eluted out with tetrahydrofuran (0.5 mL) and the solvent was removed with a stream of helium to give a residue of **8** that was used in the next step.

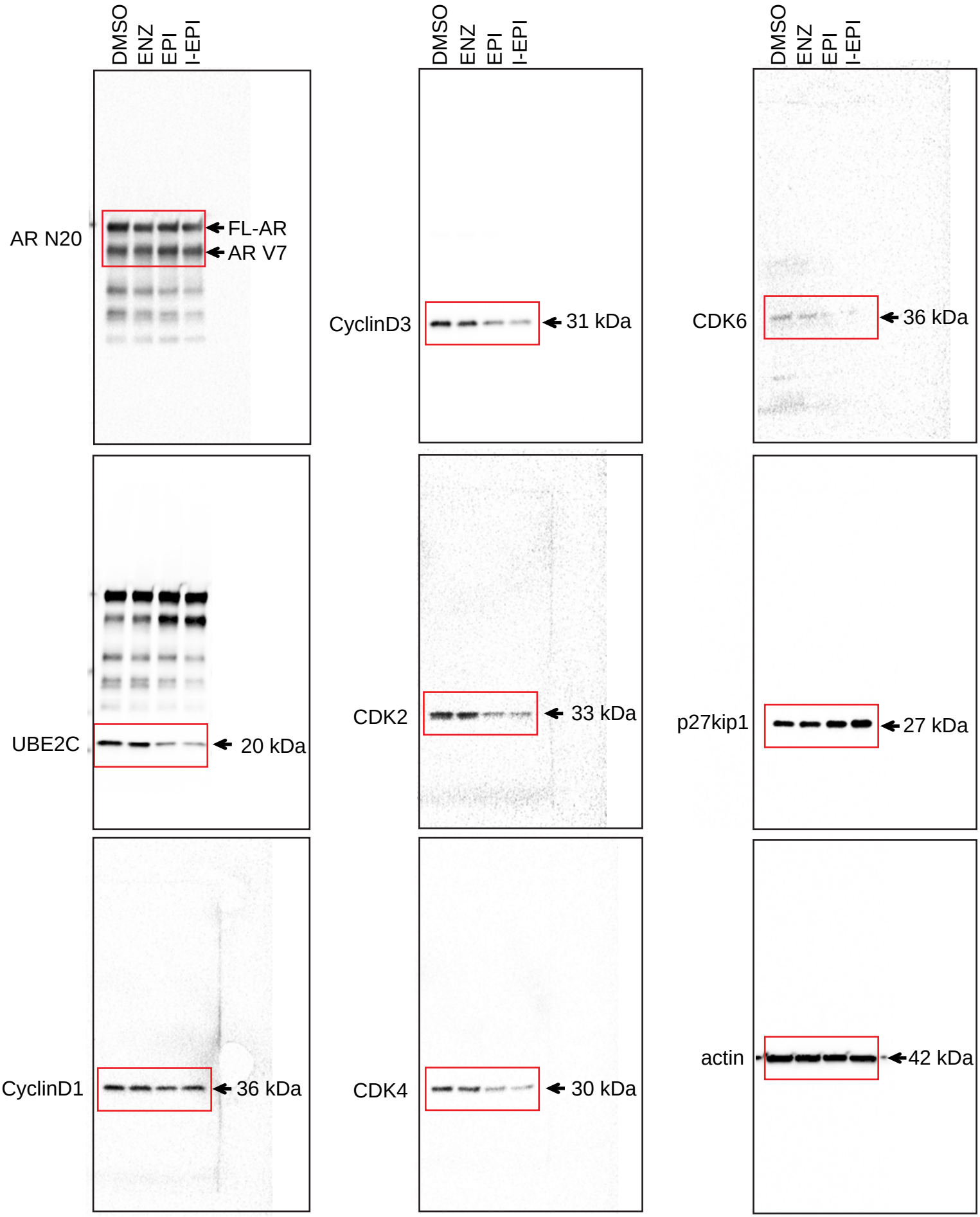
Preparation of [^{123}I]15-IodoEPI002, 9: To the above dried [^{123}I]iodoepoxide **8** was added 40 mg $CeCl_3 \cdot 7H_2O$ and 500 μL anhydrous acetonitrile. The mixture was heated at 110 °C for 60 min. At the end of heating, the mixture was diluted with water (500 μL) and purified by HPLC using the Phenomenex Luna semipreparative column eluting with 49% acetonitrile/51% water at a flow rate of 4.5 mL/min. The retention time of [^{123}I]iodoexpoxide **8** was 15.9 min. The eluting fraction containing [^{123}I]15-iodoEPI002, **9,** was collected, diluted with water (50 mL), and passed through a Waters C18 light Sep-Pak cartridge. The trapped [^{123}I]15-iodoEPI002, **9,** on the Sep-Pak cartridge was eluted out with ethanol (0.5 mL), the ethanol was removed with a helium stream, and the dried residue was dissolved in DMSO for in vitro studies or 30% PEG solution for in vivo studies. The quality control of [^{123}I]15-iodoEPI002, **9**, was performed on HPLC using the

Phenomenex Luna analytical column eluting with 50% acetonitrile/50% water at a flow rate of 2 mL/min. The retention time of [^{123}I]15-iodoEPI002, **9**, was 7.4 min. The specific activity of [^{123}I]15-iodoEPI002, **9**, was measured using the analytical HPLC system. It was calculated by dividing the injected radioactivity of [^{123}I]15-iodoEPI002, **9**, by the mass of total 15-iodoEPI002, **6** + **9**, in the injected solution. The mass of 15-iodoEPI002, **6** + **9**, was estimated by comparing the UV absorbance obtained from the injection with a previously prepared standard curve of cold 15-iodoEPI002,**6**.

Full unedited gel for Figure 3B



Full unedited gel for Figure 3F

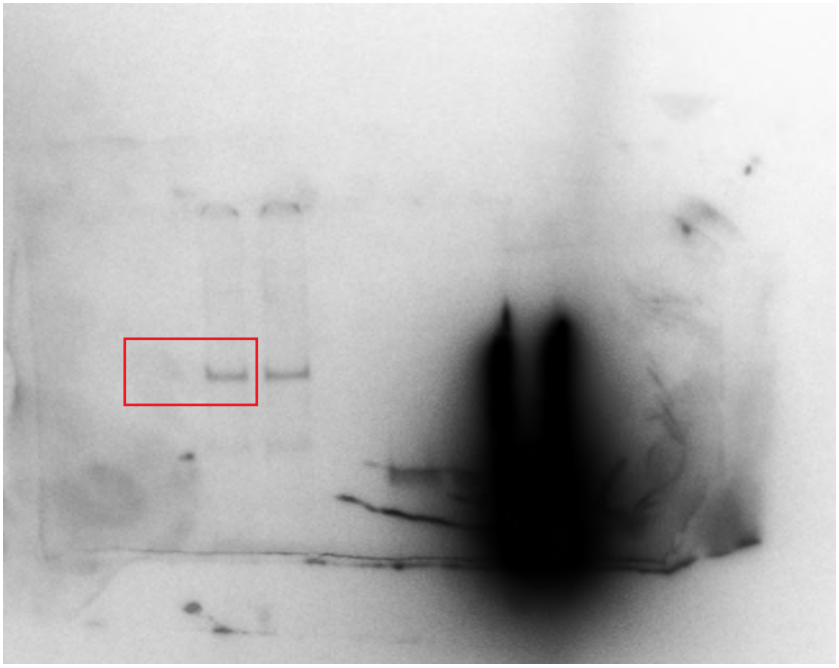


Full unedited gel for Figure 4B

FL-AR + +

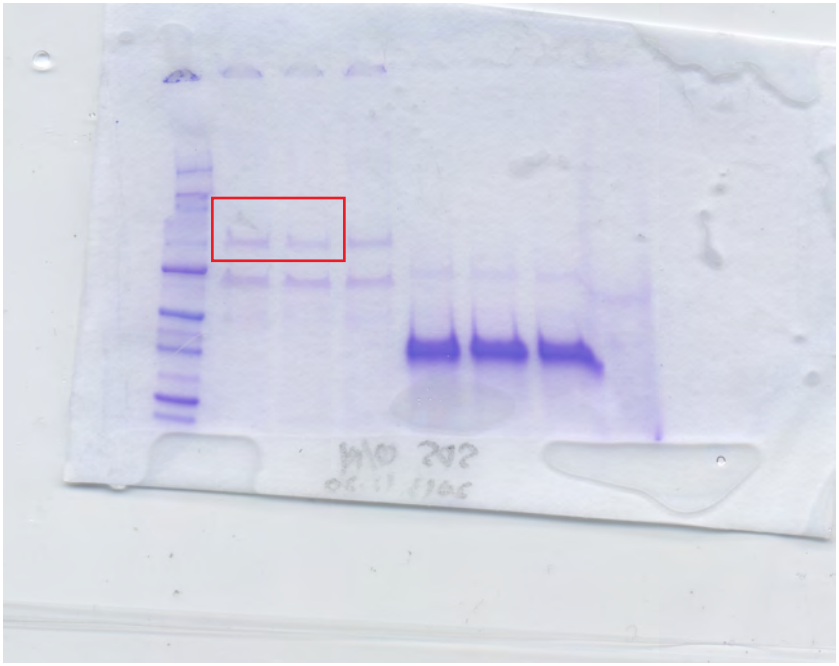
^{123}I-EPI-002 - +

Phosphorimage



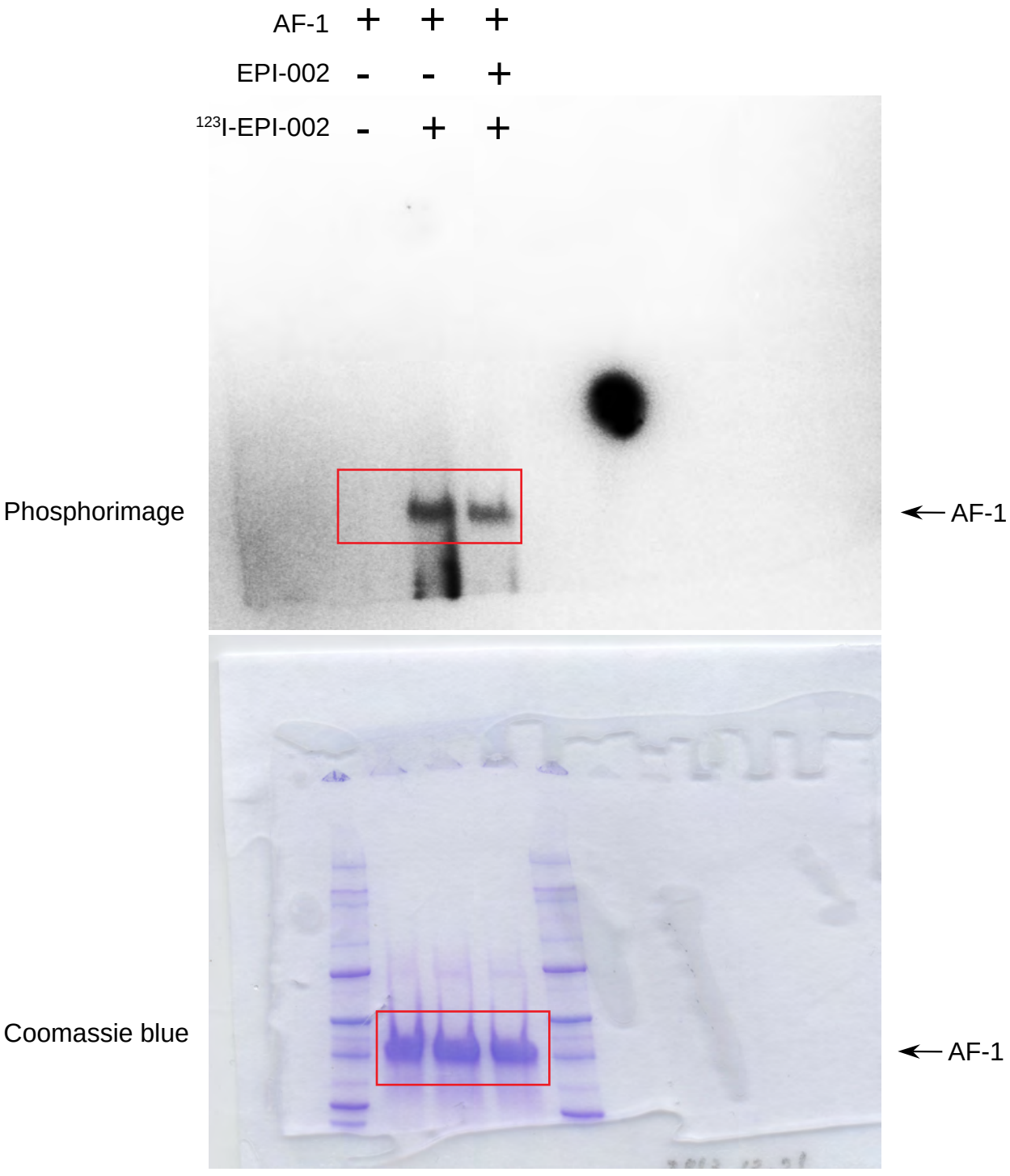
← FL-AR

Coomassie blue

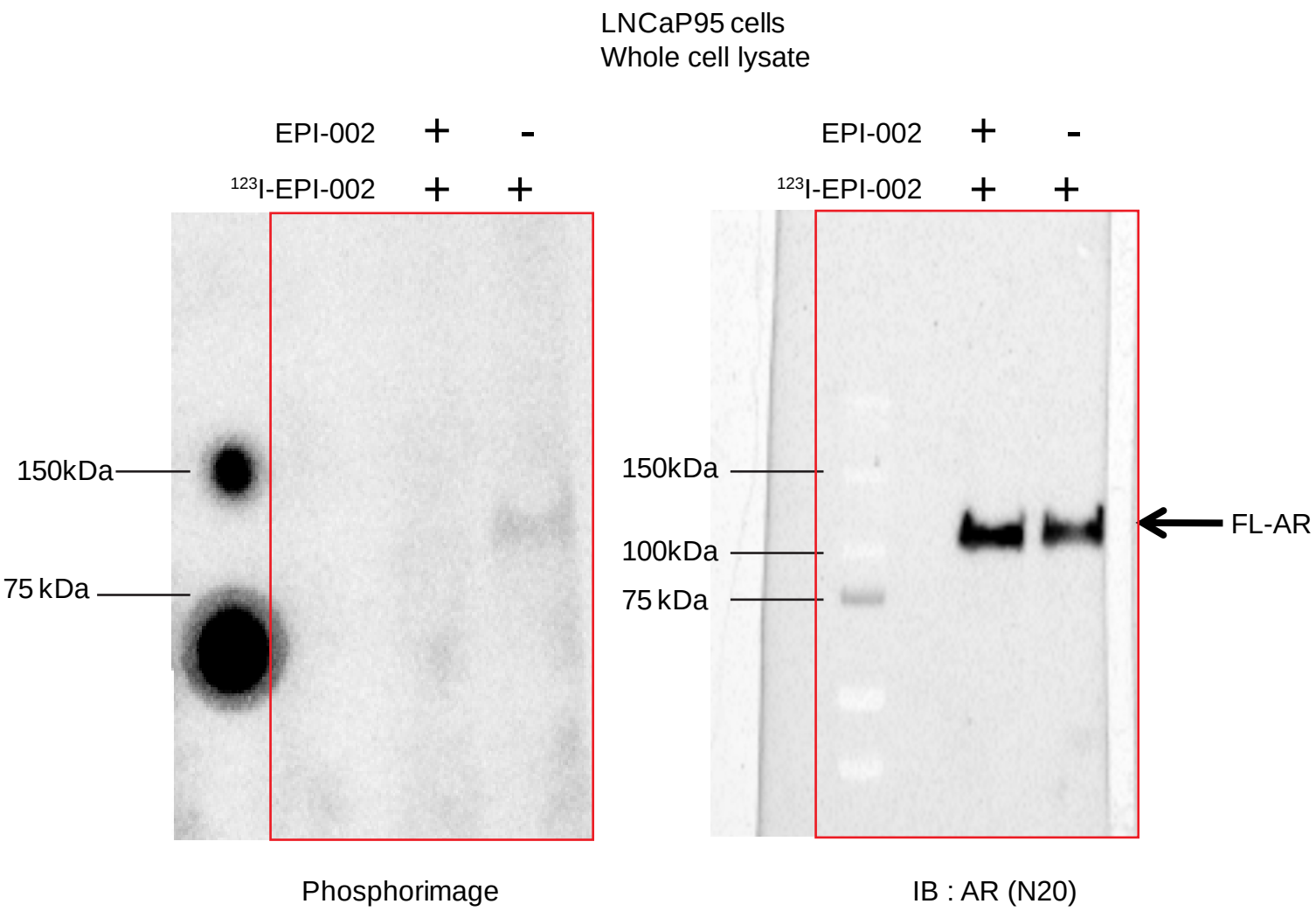


← FL-AR

Full unedited gel for Figure 4C



Full unedited gel for Figure 4D



Full unedited gel for Figure 5E