

All-trans Retinoic Acid and Sodium Butyrate Enhance Natriuretic Peptide Receptor A Gene Transcription *in vivo*: Role of Histone Modification

Prerna Kumar, Ramu Periyasamy, Subhankar Das, Smitha Neerukonda, Indra Mani, and Kailash N. Pandey

*Molecular Pharmacology*

**Supplemental Table I: List of the antibodies used in Western blot (WB), immunoprecipitation (IP), immunohistochemistry (IHC), and chromatin immunoprecipitation (ChIP) assay.**

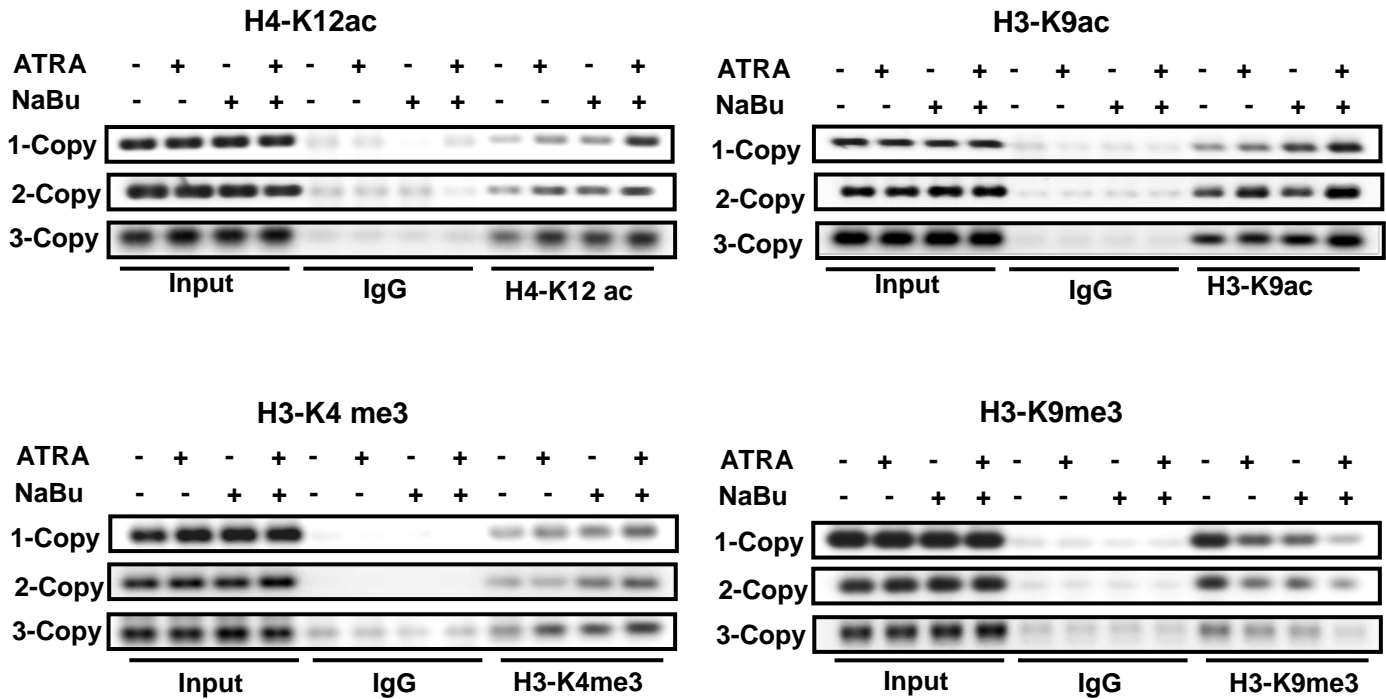
| <b>Protein</b> | <b>Reference</b> | <b>Source</b>                                     | <b>Assay</b> |
|----------------|------------------|---|--------------|
| NPRA           | 15-288-22960     | Genway Biotech Inc.<br>(San Diego, CA)            | WB           |
| H4(K-12)ac     | sc-8661-R        | Santa Cruz Biotechnology Inc.<br>(Santa Cruz, CA) | WB, ChIP     |
| H3(K-9/14)ac   | 06-599           | Upstate Biotechnology<br>(Lake Placid, NY)        | WB, ChIP     |
| H3(K-9)me3     | A-4036-050       | Epigentek<br>(Farmingdale, NY)                    | WB, ChIP     |
| H3-K4me3       | 39159            | Active Motif<br>(Carlsbad, CA)                    | WB, ChIP     |
| Ets-1          | sc-111X          | Santa Cruz Biotechnology Inc.<br>(Santa Cruz, CA) | WB, ChIP     |
| RAR $\alpha$   | sc-15040X        | Santa Cruz Biotechnology Inc.<br>(Santa Cruz, CA) | WB, ChIP     |
| p300           | A-4020-100       | Epigentek<br>(Farmingdale, NY)                    | WB, ChIP     |
| PCAF           | sc-13124         | Santa Cruz Biotechnology Inc.<br>(Santa Cruz, CA) | WB, ChIP     |
| Alpha-SMA      | A-7607           | Sigma Aldrich<br>(St. Louis, MO)                  | WB, IHC      |
| PCNA           | sc-25280         | Santa Cruz Biotechnology Inc.<br>(Santa Cruz, CA) | WB, IHC      |
| H3             | sc-10809         | Santa Cruz Biotechnology Inc.<br>(Santa Cruz, CA) | WB           |
| $\beta$ -actin | A5316            | Sigma Aldrich<br>(St. Louis, MO)                  | WB           |
| TBP            | sc-271146        | Santa Cruz Biotechnology Inc.<br>(Santa Cruz, CA) | WB           |

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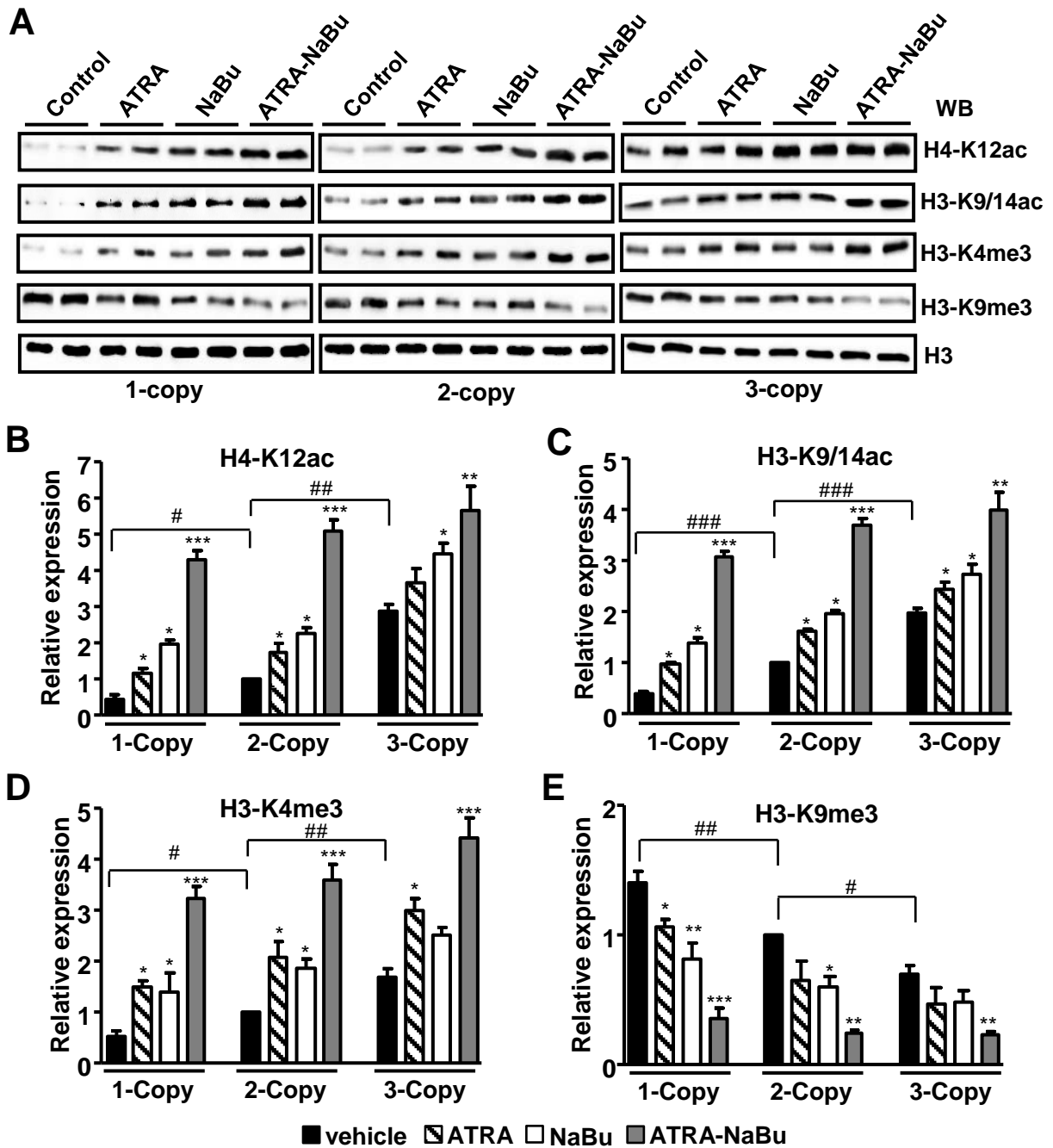
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**Supplemental Figure 1**



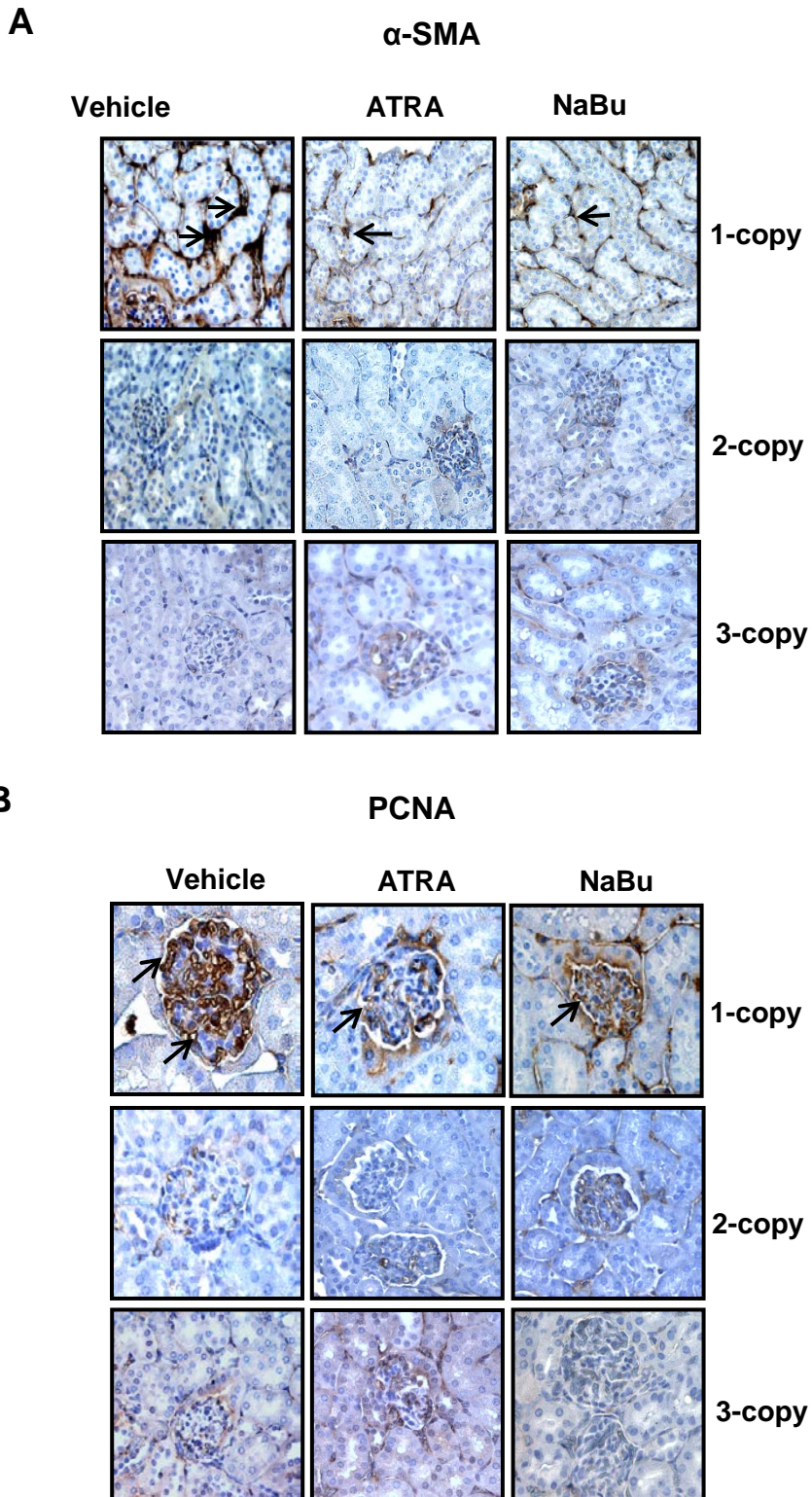
**Supplemental Figure 1. Effect of ATRA and NaBu on recruitment of modified histones at the *Npr1* proximal promoter in kidneys of *Npr1* gene-targeted mice.** Representative gel images of ChIP assay demonstrating recruitment of H4-K12ac, H3-K9ac, H3-K4me3, and H3-K9me3 to the *Npr1* promoter in kidneys of 1-copy, 2-copy, and 3-copy mice. Pooled samples from 7 mice in each group were used for ChIP and purified DNA was amplified by conventional PCR.

## Supplemental Figure 2



**Supplemental Figure 2. Renal protein expression of histone modifications in ATRA- and NaBu-treated *Npr1* gene-targeted mice.** (A) Western blot analyses of expression levels of acetylated and methylated histones in drug- or vehicle-treated *Npr1* gene-targeted mice. Densitometry analyses of H4-K12ac (B), H3-K9/14ac (C), H3-K4me3 (D), and H3-K9me3 (E) protein expression in renal tissues. Histone H3 was used as loading control. Densitometry analysis was done by AlphaInnotech phosphoimager software. Values are expressed as mean  $\pm$  SE of three independent experiments. WB indicates Western blot; \*  $p < 0.05$ ; \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  (vehicle-treated versus drug-treated same group); #  $p < 0.05$ , ##  $p < 0.01$ , ###  $p < 0.001$  (1-copy or 3-copy versus 2-copy);  $n=8$  mice per group.

## Supplemental Figure 3



**Supplemental Figure 3. Renal immunoexpression of  $\alpha$ -SMA and PCNA in drug-treated and vehicle-treated *Npr1* gene-targeted and wild-type mice.** Immunohistochemistry showing renal expression of  $\alpha$ -SMA (A) and PCNA (B) in ATRA- and NaBu-treated 1-copy, 2-copy, and 3-copy mice.