

Tissue Collection:

For Spinal Cord: Cut off head slicing through cervical spinal cord. Cut through spinal cord again just above the pelvis with sharp scissors. Insert syringe with 18 gauge needle filled with cold PBS into the spinal column at the pelvic end, pointing toward the cervical end. Using significant force, push down the plunger of the syringe until the spinal cord shoots out in one piece from the cervical end of the spinal column. Make sure there is a dish with cold PBS on the cervical end to catch the spinal cord. Wash in cold PBS and mince completely with a razor blade while on ice.

For Skin: Secure mouse face-down to a piece of Styrofoam or a dissecting pad with pins through its hands and feet, stretched out completely, but not too tight, to each corner. Liberally apply Nair (or comparable hair removal product) to a quarter-sized patch of skin on back of mouse, until hair begins to fall away. Wash extensively and continuously with cold water for at least a minute. If patch of skin is not completely hairless, repeat the process. Using sharp scissors, cut away patch of skin, being careful not to remove any underlying tissues. Cut up as much as possible with scissors in cold PBS and finish mincing with razor blade while on ice. Skin is difficult to mince but must be minced completely for successful experiments.

For Stomach: Cut into the abdominal cavity following the same line you cut along through the skin. Pull stomach forward with forceps and cut through the esophageal and pyloric sphincters (where organ narrows on either end) with sharp scissors. Cut organ down the middle on one side to completely expose the stomach contents. Completely wash away contents with cold PBS. Wash again with cold PBS and mince completely with a razor blade while on ice.

For Thigh Muscle (Quadriceps): Using your hands, pull skin back over the legs, grabbing the quadriceps to pull the legs up through the skin, exposing them completely. The skin may also be cut away completely from the legs if needed. Using sharp scissors, poke through the leg perpendicularly just above the femur and open the scissors laterally to widen the hole. Cut on either end of widened hole, perpendicularly, to remove the quadriceps muscle. Wash with cold PBS and mince completely with a razor blade while on ice.

Tissue Preparation:

Minced tissues were cross-linked in PBS with 1/10 volume of cross-linking buffer (0.1M NaCl, 1mM EDTA, 0.5mM EGTA, 50mM Hepes pH 8.0, 11% Formaldehyde) for 25 minutes on a rotating platform and quenched for 5 minutes by adding Glycine to a final concentration of 0.125 M. The samples were then centrifuged at 2500 RPM for 5 minutes at 4°C, supernatant discarded, washed once with cold PBS, and centrifuged again. The tissue pellets were re-suspended in 1mL of cold PBS and homogenized with a Wheaton tight stroke 1mL, dounce homogenizer. If multiple tissues from separate animals needed to be combined to attain the required 0.1g to 0.3g of tissue required per ChIP, they were combined at this time, so that all of these tissues to be combined would be resuspended in the same 1mL of cold PBS. After homogenization, the solution was transferred to a 2mL microcentrifuge tube and centrifuged at 2500 RPM for 5 minutes at 4°C. After discarding the supernatant, the tissue pellet was flash frozen on dry ice and then stored at -80°C.