Detailed description of necropsy procedures for RaDR mice

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Necropsy and sample collection

1. Euthanize mouse according to AVMA guidelines, your IACUC-approved protocol, and institutional guidelines.

2. Remove mouse from chamber and place on clean workspace. After confirming death, place the mouse supine on a corkboard (or other surface that can hold pins), extend its limbs, and pin down.

3. Spray the mouse's belly with 70% ethanol and smooth down fur. This will help prevent fur from flying and sticking to instruments. Mouse fur is autofluorescent and will interfere with fluorescence imaging of RaDR tissues, so it is best to minimize its contact with tissues and instruments whenever possible.

4. Using a scalpel, split the mouse's skin down the center and make incisions toward the limbs. Carefully separate the skin from the peritoneum, stretch it to either side, and pin down.

5. Collect tissues of interest. It is recommended that you work in the order below for ease of collection. Hold all tissues on ice until ready to use. All tissues can be processed for flow cytometry (see protocol Preparing Mouse Tissues for Flow Cytometry). *Starred tissues can be imaged for RaDR foci on a fluorescent microscope.

    a. ***Mammae** – separate mammary tissue from the skin with a scalpel. Breast tissue is sticky, and it is very difficult to remove fur once it has come in contact with breast tissue, so wipe the scalpel off on a paper towel between strokes. Hold in PBS on ice.

    b. ***Pancreas** – the pancreas is located between the stomach and intestines. It is soft, pale pink, and amorphous. Using scissors, separate the pancreas from the spleen, intestines, mesentery, and stomach. Place in 0.01% trypsin inhibitor diluted in PBS and hold on ice.

    c. **Spleen** – the spleen is a thin, long, dark red organ on the mouse's left side. There is a thin membrane connecting it to the stomach that can be trimmed easily. Hold in PBS on ice.

    d. ***Liver** – the mouse liver is composed of four lobes – the left is the largest and most central in the abdomen; the medial lobe has the gall bladder protruding from its center; the right lobe is smaller and slightly behind the medial, and the caudal

lobe is behind the others. In the Engelward lab, we usually collect the left lobe for imaging, but all lobes can be imaged or processed for flow cytometry. These can be excised with forceps and scissors. Hold in 0.01% trypsin inhibitor in PBS on ice.

e. ***Intestines** – the intestines are held in place with a web of mesentery. Mesentery is autofluorescent, so it should be trimmed away as completely as possible. To begin, use forceps to grasp the colon near the rectum. Gently pull up and snip the colon as close to the anus as possible. Gently pull the colon away from the body, letting the intestines unravel and mesentery detach. Depending on the mouse strain and treatments, the intestines may be fragile, so be careful to avoid tearing the tissue. Use scissors to snip away mesentery and facilitate unraveling. To remove the remaining mesentery, stretch the intestine out on a paper towel – the mesentery will stick to the paper as you gently roll the intestine to either side and you can snip or slice it away.

   The colon and small intestine are connected at a sac called the cecum, which can be imaged as well. The small intestine is composed of three segments called the ileum (distal, attached to the cecum), jejunum (medial), and duodenum (proximal, connected to the stomach). After the desired segments have been excised, cut through one side of the intestine tube longitudinally to open the lumen. Gently clean out the contents of the intestines with PBS. Save in .01% trypsin inhibitor in PBS and hold on ice.

   Note: normal mouse chow contains alfalfa, which fluoresces at the same wavelength as EGFP. If the mice have eaten normal food, the intestines will need to be thoroughly washed of all particulates, which can disturb the architecture of the lumen. To prevent interference of food particles, we recommend feeding the mice a special non-fluorescent diet, such as AIN-93G, for at least one week prior to imaging. Intestines should still be rinsed of particulates before imaging, but this does not need to be as thorough if the food is non-fluorescent.

f. **Stomach** – excise the stomach and rinse out contents with PBS. Hold in .01% trypsin inhibitor on ice.

g. **Lungs** – once lower abdominal organs of interest have been removed, use scissors to cut open the mouse's ribcage. Lungs are bright pink and composed of one left lobe and several right lobes. Hold in PBS on ice.

h. **Heart** – remove with scissors, hold in PBS on ice.

i. **Thymus** – the thymus is a small gland with two lobes located directly above the heart, in the mouse's throat. Pull down with forceps, excise with scissors, save in PBS and hold on ice.

j. **Brain** – once all other tissues have been excised, unpin the mouse and flip over. Spray the mouse's head with 70% ethanol and cut the skin open with a scalpel. Secure the mouse's skull with forceps, orient the scalpel down the center, and push down firmly to break open. Carefully remove the brain and hold in PBS on ice.

Imaging

1. Compress tissue between coverslips and tape together at edges.

    a. **Pancreas** tissue is very amorphous, so to maintain an even surface we use spacers with a depth of 0.5mm. Place on spacer on either side of the pancreas before placing the second coverslip on top and taping.

    b. **Liver** and **mammary** tissues are thick and will cause slight warping of the coverslips after taping. Do not use spacers, as they will not be held securely and fall out due to the thickness of the tissue.

    c. **Intestines** should be gently spread open into a single layer with a wet cotton applicator. Intestines are usually even thickness, and thinner than 0.5mm, so spacers are not necessary. If there are large tumors in the intestines, they may squeeze out the side of the coverslip sandwich. In this case, it helps to use spacers to widen the space between the coverslips to accommodate tumors.

2. Tissues are imaged on the FITC channel of a fluorescence microscope. We typically image on a 1x or 2x objective and scan the entire area of the tissue. Our microscope software stitches the images together, but this can also be accomplished manually.

3. We report RaDR foci data as the number of foci per unit area. The area of the tissue can be measured in ImageJ by opening the microscope image, setting the scale according to your microscope objective's distance:pixel ratio, tracing the outer edge of the tissue, and measuring the area. Foci can be counted manually or with an image processing program.